ASTMH
AMERICAN SOCIETY OF TROPICAL MEDICINE & HYGIENE
ADVANCING GLOBAL HEALTH SINCE 1903

VOLUME 115 NOVEMBER 2026 NUMBER 5 SUPPLEMENT

Abstract Book

ASTMH Annual Meeting

TropMed26

November 18-22, 2026

Gaylord National Harbor, MD, USA



Advancing Science for Tropical Medicine and Health

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CELLS AND MOSQUITOES AND MICE, OH MY! - PERSPECTIVES FROM INFECTION STUDIES WITH BUSSUQUARA VIRUS

Madeline R. Steck[1], Marielena Vogel Saivish[1], Cecília A. Banho[2], Vsevolod L. Popov[1], Haiping Hao[1], Gabriela L. Menezes[3], Umberto L. Fulco[3], Roosevelt A. da Silva[4], Kathryn A. Hanley[5], Mauricio L. Nogueira[6], Shannan L. Rossi[1], Nikos Vasilakis[1]

[1]University of Texas Medical Branch, Galveston, TX, United States, [2] Faculdade de Medicina de São José do Rio Preto, São José do Rio Preto, Brazil, [3]Universidade Federal do Rio Grande do Norte, Natal, Brazil, [4]Universidade Federal de Jataí, Jatai, Brazil, [5]New Mexico State University, Las Cruces, NM, United States, [6]Faculdade de Medicina de Sao Jose Do Rio Preto, SJ Rio Preto, Brazil

Bussuquara virus (BSQV) (*Flaviviridae, Orthoflavivirus*) is a neotropical mosquito-borne virus first isolated in Brazil, 1956. BSQV is neglected in contemporary surveillance and clinical institutions despite historical implications of a broad vector-host range, broad geographic circulation, and neurotropism. Our objective was to assess BSQV emergence potential in the Americas by investigating (i) orthoflavivirus biology, (ii) *in vitro* infection kinetics and cytopathology, (iii) *in vivo* vector competence, (iv) animal model pathogenesis, alongside (v) building capacity for sensitive and specific BSQV detection. We utilized three historical BSQV isolates and leveraged diverse techniques including next generation sequencing, genome annotation, transmission electron microscopy, phylogenetic tree construction, replication kinetics in mosquito (*Aedes, Culex*) and vertebrate (human, non-human primate, rodent, marsupial, avian) cells, artificial and live bloodmeal inoculation of domestic and peri-domestic mosquito species (*Aedes, Culex*), optimization of molecular (RT-qPCR) and serological (ELISA) diagnostic assays, and development of BSQV murine animal models with histopathology and viral load analyses. Our data builds premise that BSQV is (i) a generalist arbovirus with a broad range of susceptible vertebrate species, (ii) largely refractory in non-sylvatic *Aedes* and *Culex* mosquitoes, (iii) capable of neuroinvasion and neurovirulence in mice.

6001

A COMMERCIAL INACTIVATED EASTERN EQUINE ENCEPHALITIS VIRUS VACCINE PROTECTS MICE AGAINST DISEASE CAUSED BY INFECTION WITH MADARIAGA VIRUS THROUGH NON-NEUTRALIZING ANTIBODY IMMUNITY

Samuel Cerezo, Shi-Hsuan Sung, Wendy Tang, Xiao Liang, Gabriel Hamer, Raquel Rech, **Tereza Magalhaes**

Texas A&M University, College Station, TX, United States

Madariaga virus (MADV), a member of the eastern equine encephalitis virus (EEEV) complex, is a mosquito-borne virus capable of causing severe neurological disease in equids and humans. MADV is endemic to Latin America but has been expanding geographically, representing an emerging threat. Because no licensed MADV vaccines exist, vaccines targeting the North American EEEV (NA-EEEV), a genetically closely related virus, could potentially be used to mitigate outbreaks involving MADV. Nevertheless, the level of cross-protection conferred by NA-EEEV vaccines against MADV remains unclear. We evaluated whether a commercial inactivated equine NA-EEEV vaccine (Prestige 3®) provides cross-protection against MADV in mice. NIH Swiss mice were assigned to three groups: (1) Prestige 3®-vaccinated and mock-challenged; (2) mock-vaccinated and MADV-challenged; or (3) Prestige 3®-vaccinated and MADV-challenged. Animals received two vaccine doses administered two weeks apart, and were challenged with 10,000 plaque forming units of virus two weeks after the booster dose. Following challenge, animals were monitored daily for 12 days for clinical signs, and blood samples were collected longitudinally. Upon euthanasia, multiple tissues were collected, including those of the central nervous system. MADV RNA and infectious virus were quantified in all biological samples, and anti-MADV neutralizing antibodies (nAbs) were measured in sera. Results showed that unvaccinated, challenged mice developed significantly more severe disease and higher mortality than vaccinated, challenged animals, demonstrating vaccine-mediated protection. Nevertheless, vaccinated, challenged mice exhibited mild clinical signs, indicating non-sterilizing protection. Meningitis and meningoencephalitis, and detectable MADV particles in multiple tissues were observed exclusively in unvaccinated, challenged mice. Anti-MADV nAb titers were unexpectedly low in vaccinated, challenged animals compared with unvaccinated, challenged mice, suggesting that protection was largely independent of nAbs.

6002

GENOMIC CHARACTERIZATION AND TRANSMISSION DYNAMICS OF THE FIRST CASES OF THE 2025 MARBURG VIRUS OUTBREAK IN ETHIOPIA

Betselot Zerihun Ayano[1], Marburg Virus Outbreak Task Force[1], Dawit Wolday[2]

[1]Ethiopian Public Health Institute, Addis Ababa, Ethiopia, [2]McMaster University, Ethiopia

In November 2025, Ethiopia reported its first Marburg virus disease (MVD) outbreak, with a case fatality rate of 64%. We characterized the genomic diversity, transmission dynamics, and mutations relevant to antiviral drug, vaccine, and monoclonal antibody efficacy. MVD diagnosis was confirmed by RT-PCR, and positive samples underwent fullgenome sequencing. Seven highquality genomes (genome coverage >70%) from the Ethiopian outbreak were successfully sequenced and analyzed alongside 103 global genomes derived from humans and the natural bat host, *Rousettus aegyptiacus*. The Ethiopian sequences showed >99.9% nucleotide identity, indicating low viral diversity. Maximum-likelihood phylogenetic analysis demonstrated that all sequences clustered within the MARV.A.1 clade. They were closely related to historical isolates from the Netherlands (the Leiden strain) and Uganda. The Ethiopian lineage and its closest human isolate (JN408064) diverged from a common ancestor circulating in 1994, whereas the Ethiopian lineage and its closest bat isolates (JX458853 and JX458858) diverged from a common ancestor circulating around 1989. Genomic and epidemiological evidence supported a single zoonotic spillover followed by localized human-to-human transmission. Seventy lineage-defining substitutions were identified, including 10 non-synonymous mutations primarily in the glycoprotein gene, with strong purifying selection (dN/dS < 1). The outbreak strain showed high similarity to the reference strain, with conserved monoclonal antibody binding sites and no escape mutations. Overall, the outbreak resulted from a single spillover event with limited viral diversification, supporting the continued efficacy of existing vaccines and monoclonal antibody therapies.

6003

SUSTAINED PEDIATRIC BURDEN AND TRANSMISSION DYNAMICS OF MPOX IN THE DEMOCRATIC REPUBLIC OF THE CONGO, 2017-2025

Junior Bulabula-Penge[1], Eddy Kinganda-Lusamaki[2], Wataru Kagaya[1], Gradi Luakanda-Ndelemo[2], Élisabeth Pukuta[2], Yannick Tutu Tshia N'kasar[3], Adrienne Amuri-Aziza[2], Antoine Nkuba-Ndaye[2], Placide Mbala-Kingebeni[2], Steve Ahuka-Mundeke[2], Satoshi Kaneko[1]

[1]Institute of Tropical Medicine (Nekken), Nagasaki University, Nagasaki, Japan, [2]Institut National de Recherche Biomédicale, Kinshasa, Democratic Republic of the Congo, [3]Ministère de la Santé Publique, Hygiène et Prévoyance Sociale, Kinshasa, Democratic Republic of the Congo

Mpox remains an ongoing threat in the Democratic Republic of The Congo, with a substantial yet undercharacterized burden among children. Limited understanding of their role in transmission may contribute to their underrepresentation in priority control strategies, particularly in remote areas where they are most affected. This study aimed to assess the burden of pediatric mpox and characterize age-specific transmission dynamics using integrated epidemiological and genomic data. We conducted a retrospective analysis of national laboratory surveillance data collected in the DRC from 2017 to 2025 through the Institut National de Recherche Biomédicale. Among 54698 individuals tested, 26674 laboratory-

confirmed cases were identified, of which 11810 (44.3%) occurred in children under 15 years of age. Children accounted for a substantial proportion of confirmed cases, exceeding 50% in earlier years (54.8% in 2017-2021) and remaining consistently high (>40%) in recent years, indicating a sustained pediatric burden despite a shift toward older age groups. Temporal trend analyses (Mann-Kendall test) showed a significant increase in mpox incidence in both age groups (children: τ=0.28, p<0.001; adults: τ=0.35, p<0.001). Change-point analysis (Pettitt test) identified an earlier shift among adults (June 2022) compared to children (December 2023), indicating a delayed but substantial increase in pediatric cases. Spatial analyses of incidence showed expansion of pediatric cases from northwestern endemic provinces to eastern regions, particularly Sud-Kivu and Nord-Kivu, after 2023. Among 2704 sequenced cases, clade Ia predominated (68.4%) and was strongly associated with children (90.1%), while clade Ib was more frequent in adults. Phylogenetic analyses showed no age-specific clustering, suggesting shared transmission dynamics between age groups. These findings highlight the sustained burden of pediatric mpox in the DRC and the need for strengthened surveillance and targeted control strategies

6004

RE-EMERGENCE AND CHARACTERIZATION OF ST. LOUIS ENCEPHALITIS VIRUS IN COLORADO

Emily Gallichotte, Kate Kimball, Marina Fujii, Gregory D. Ebel
Colorado State University, Fort Collins, CO, United States

St. Louis encephalitis virus (SLEV) is a single-stranded, positive sense RNA virus in the family Flaviviridae. It is transmitted by Culex mosquitoes, with birds as the primary vertebrate host. SLEV was endemic throughout the entire United States until the early 2000s, but was displaced by West Nile virus (WNV) in 2002-2003. In 2014, SLEV was reintroduced to the southwestern US from Argentina, and has been circulating in that region since. Although SLEV historically caused both human and veterinary infections in Colorado, it has not been detected in several decades, however has circulated in neighboring states since 2017. In September 2025, we identified five SLEV-positive mosquito pools (Cx. tarsalis and Cx. pipiens) in Northern Colorado. The viral envelope ectodomain was sequenced and compared to current and historic strains. While all Colorado SLEV sequences are genotype 3, some group with the contemporary SLEV that circulates in CA and AZ, however others are more closely related to SLEV from Argentina in 2005-2024. Infectious virus was recovered from a Fort Collins 2025 mosquito pool (FC'25) and compared to other historic and contemporary genetically diverse SLEV isolates. FC'25 generated significantly smaller plaques compared to other genotype 2 and 3 strains, however all viruses replicated similarly in chicken fibroblast and Jamaican fruit bat kidney cells. In Cx. tarsalis cells, replication was more variable across isolates, however FC'25 reached significantly higher titers than a related genotype 3 Arizona 2015 strain. Serum from 2025 wild-caught birds will be tested for the presence of neutralizing antibodies against SLEV to estimate levels of infection in avian hosts. Additionally, serum from birds experimentally infected with a historic genotype 2 virus will be tested for neutralizing breadth against FC'25 and other contemporary genotype 3 strains. This work will identify genotypic and phenotypic differences between the viruses currently present in northern Colorado and surrounding states to historic viruses, which may provide insight into its re-emergence after 20+ years.

6005

EVOLUTIONARY AND STRUCTURAL LINKS BETWEEN THE NEGLECTED INGWAVUMA VIRUS AND THE PATHOGENIC OROPOUCHE VIRUS REVEAL CONSERVED GLYCOPROTEIN REGIONS.

Safietou Sankhe[1], Walid Heiba[2], Ndeye Awa Ndiaye[1], Mouhamed Kane[1], Joseph R. Fitchett[1], Ibrahima Socé Fall[1], Ndongo Dia[1], Moussa Moise Diagne[1]
[1]Institut Pasteur de Dakar, Dakar, Senegal, [2]University Alexandria, Caire, Egypt

Characterizing neglected viruses is essential for preparedness, enabling rapid vaccine development when outbreaks occur. Orthobunyaviruses are tri-segmented RNA arboviruses transmitted by arthropods and capable of infecting humans and animals. However, Ingwavuma virus (INGV), despite its close phylogenetic relationship to the pathogenic Oropouche virus (OROV), remains poorly characterized. Only two complete INGV genome sequences exist globally, highlighting a major knowledge gap and limiting our understanding of its evolution, and potential for emergence. Here, we combined phylogenetic analyses and structure-guided comparative analysis to elucidate the relationship between pathogenic OROV and uncharacterized INGV and identify conserved glycoprotein regions potentially involved in viral entry. Our results showed that OROV and INGV form a sister lineage that diverged in the mid-18th century with a median time to the most recent common ancestor around 1758 and a 95% highest posterior density interval spanning from 1575 to 1895. Structural modeling of glycoprotein N-terminal and C-terminal regions revealed highly conserved domains at the Gn-Gc interface and within the fusion loops, highlighting candidate conserved functional constraints and hypothesis-generating regions for future experimental mapping (e.g., receptor binding and neutralization assays). This framework linking structural insights advances our understanding of neglected Orthobunyaviruses and prioritized candidates for the development of cross-reactive antiviral concepts and future vaccine strategies.

6006

ADVANCING LASSA VIRUS DETECTION: INTEGRATED SEROPREVALENCE AND MOLECULAR DIAGNOSTICS IN ILORIN, NIGERIA

Olatunji Matthew Kolawole[1], Damilola Miracle Olayemi[2], Olayinka Idris[3], Olumuyiwa Babalola Salu[4], Sunday Aremu Omilabu[4], Ilaria Dorigatti[5]
[1]University of Ilorin, Ilorin, Nigeria, [2]KW, Nigeria, [3]Afe Babalola University, Ekiti, Nigeria, [4]University of Lagos, Lagos, Nigeria, [5]Imperial College, London, United Kingdom

Lassa fever remains a major viral hemorrhagic threat in West Africa, with Nigeria bearing the highest burden and case fatality rates of 15-50% among hospitalized patients. Current diagnostic approaches are constrained by poor sensitivity across viral lineages, serological cross-reactivity, and fragmented testing strategies that undermine timely detection and outbreak control. A cross-sectional study of 250 febrile patients at Sobi Specialist Hospital, Ilorin, Nigeria evaluated an integrated serological-molecular diagnostic approach, with serum samples analyzed using IgM and IgG ELISA and standardized optical density thresholds, while molecular detection of Lassa virus RNA was performed using one-step RT-PCR targeting the S segment with confirmation by 320 bp amplicons; 28 samples were prioritized for molecular analysis based on serological reactivity. Seroprevalence analysis revealed IgM positivity in 16% and IgG in 8% of participants, with 2% dual seropositivity indicating recent infection, alongside a significant increase with age (p<0.001). RT-PCR confirmed active infection in 10.7% of highly seropositive samples, with positive cases demonstrating elevated IgM and IgG optical density values, supporting a strong association between serological reactivity and active viral detection. These findings indicate substantial underrecognition of Lassa fever exposure in urban populations and identify seropositive individuals with molecular confirmation as potential transmission reservoirs requiring

immediate intervention. Overall, integrating serological and molecular diagnostics enhances the detection of Lassa virus infection in endemic settings, providing a scalable framework for improving surveillance sensitivity, accelerating case identification, and strengthening outbreak response.

6007

VIRAL INFECTION KINETICS AND IMMUNE RESPONSE OF WEST NILE VIRUS AND RECENTLY RE-EMERGED ST. LOUIS ENCEPHALITIS VIRUS IN COLORADO AVIAN RESERVOIR HOSTS

Jennifer Buczek[1], Emily Gallichotte[1], Gregory D. Ebel[1], Lark L. Coffey[2], Angela Bosco-Lauth[3]

[1]Colorado State University, Fort Collins, CO, United States, [2]University of California Davis, School of Veterinary Medicine, Davis, CA, United States, [3]Colorado State Univ, Fort Collins, CO, United States

St. Louis Encephalitis virus (SLEV) is a zoonotic mosquito-borne flavivirus that re-emerged in the southwestern United States in 2015 and was recently isolated in Colorado (summer 2025). SLEV co-circulates with another mosquito borne flavivirus, West Nile virus (WNV) using the same avian vertebrate hosts (passerines). Prior to SLEV re-emergence, WNV seemingly outcompeted endemic SLEV strains in 2003 and due to some unknown factor(s) contemporary strains of SLEV (cSLEV) have escaped viral competition to re-establish endemicity. Currently, infection dynamics between WNV and cSLEV are unknown. Our hypothesis is that cSLEV effectively evades avian reservoir host immunity such that prior infection with WNV does not protect against cSLEV infection. To investigate infection dynamics in Colorado, we performed heterologous virus infections in wild-caught house sparrows (Passer domesticus) (n=20) using the newly emerged Colorado SLEV isolate and a WNV strain also isolated from Colorado. Birds were divided into 2 groups (n=10) and challenged with WNV or SLEV. After 28 days, surviving birds were challenged with reciprocal virus. Viremic levels (days 1-5, 7), clinical signs, mortality, and neutralizing antibody were recorded for each bird. Birds infected first with WNV were aviremic after SLEV challenge demonstrating complete cross-neutralizing protection in the short term. Whereas in group 2, WNV breaks through SLEV immunity and produces similar viremic curves as primary WNV infected birds. Interestingly, one colorado bird with natural SLEV immunity further challenged with SLEV and then WNV subsequently had a delayed and 5-log higher peak viremia with mortality after 4 days post-infection. These findings mirror those observed between a Californian cSLEV strain and WNV suggesting that there may be an SLEV antibody-dependent enhancement of WNV replication. Overall, this study illustrates the complexity of flaviviral infection kinetics, strain variability, and avian immunity which requires further investigation.

6008

MECHANISMS CONTRIBUTING TO GROWTH FAILURE IN GIARDIASIS

Steven Singer[1], Rita Kosile[2], Bergeline Clariste Nguemwo Tentokam[3], Vanessa V. Angelova[1], Nikki Farnham[1], Peiran Shen[1]

[1]Georgetown University, Washington, DC, United States, [2]Georgetown University, Rockville, MD, United States, [3]Germantown, MD, United States

Giardiasis is a widespread protozoan infection leading to diarrhea and growth failure, especially in children in low- and middle-income countries. Many studies have identified epithelial barrier defects due to *Giardia* infection, although mechanisms resulting in barrier defects are not well-described. We began by investigating the role of protease activated receptor (PAR)-2 signlaing on epithelial cells in producing barrier defects. Data with Caco2 cell lines show that Giardia induces barrier disfunction via PAR-2 mediated signaling. Ongoing studies are defining the parasite proteases mediating these signals. Recent studies have also suggested that signaling through the aryl hydrocarbon receptor (AhR) can promote barrier repair, so we investigated whether AhR mediated signaling could promote growth in giardiasis. While mice fed standard diets do not exhibit growth failure when infected with *Giardia*, mice fed a diet with reduced calories derived from protein (and replaced with carbohydrate) display infection-dependent growth failure along with intestinal barrier defects. Groups of mice were placed on custom diets for 1 week and then infected with *Giardia* strain GS or a luciferase tagged strain developed in our lab. Weights and infection burden were monitored for 3 weeks. Our data show that mice fed LPD had reduced plasma levels of three indole compounds known to activate AhR and that supplementation with Trp increased these levels. Supplementation with Trp also eliminated infection-dependent growth failure and increased mRNA levels of the AhR target gene Cyp1a1. While mice fed LPD had higher parasite burdens than mice fed a standard diet, Trp supplementation did not impact parasite clearance, suggesting that effects on barrier function rather than immune responses are mediating growth failure. These data suggest that diets rich in Trp may benefit children with giardiasis and potentially other enteric infections associated with growth failure.

6009

DELINEATING CELL TYPE-SPECIFIC CLEARANCE OF *TRYPANOSOMA CRUZI*

Dominic A. Ritacco, Ryan G. Gaudet

Columbia University Irving Medical Center, New York, NY, United States

Chagas disease, caused by *Trypanosoma cruzi* parasites, affects 6-8 million people worldwide and has recently been declared endemic in the United States. Pathogenesis is hallmarked by an acute pleotropic infection, a sharp decline in parasitemia, and the establishment of chronic muscle tropism, resulting in life-threatening cardiac and gastrointestinal complications. Why can these microbes be eradicated from some cell types and not others? Both mouse models and human patients have shown that interferon-gamma (IFN-γ) is the crucial host factor in controlling *T. cruzi* infection, though the downstream effectors are unknown. Recent breakthroughs have revealed that this cytokine induces elaborate interferon-stimulated gene (ISG) programs that direct antipathogen responses in a remarkably cell type- and tissue-specific manner. We hypothesize that IFN-γ-induced, cell type-specific host dense programs underlie the selective clearance and muscle tropism of *T. cruzi*. To test this, we assessed IFN-γ-activated immunity in human primary cells representing targets of acute (macrophages, endothelial cells) and chronic (smooth, skeletal, and cardiac muscle) infection using GFP-expressing *T. cruzi*. Strikingly, acute targets robustly clear parasites, whereas chronic targets only partially restrict or are entirely permissive to infection. Whole-transcriptome profiling revealed differential ISG signatures across cell lineages, and deletion of several ISG-encoded effectors expressed specifically in acute targets impaired parasite clearance. We also found that despite the ISG *IDO1* being upregulated in all cell types, it exhibited lineage-specific functions: in muscle, IDO1 catabolized tryptophan to limit parasite growth but failed to clear infection; in endothelial cells, IDO1 mediated early parasite clearance via a novel tryptophan-independent mechanism. These findings highlight the ability of ISG programs to govern parasite tropism and persistence. Moreover, they could inform intervention strategies for infections where therapeutics fail to resolve pathogen persistence, such as Chagas disease.

6010

IMMUNE PROFILING OF NATURALLY *TRYPANOSOMA CRUZI*-INFECTED RHESUS MACAQUES THROUGH SINGLE CELL TRANSCRIPTOMICS

Rachel Maria Clear[1], Weihong Tu[1], Preston Marx[1], Kelly Goff[2], Claudia P. Herrera[1], Eric Dumonteil[1]

[1]Tulane University, New Orleans, LA, United States, [2]Tulane National Biomedical Research Center, Covington, LA, United States

Chagas Disease leads to cardiomyopathy in about 1/3 of persons. Although pathophysiology is complex, and not fully elucidated, it is known that a dysregulated immune response contributes to cardiomyopathy progression. A cohort of chronically, naturally infected macaques was divided into three phenotypes: A) progressors with a proinflammatory

environment, B) progressors with a more regulated environment, and C) controllers of disease. PBMCs from this cohort and uninfected macaques underwent single cell RNA sequencing to better decipher the immune cell types and gene pathways associated with each phenotype. Progressor A macaques exhibited a highly differential immune profile to both progressor B and controller Cs; fewer significant differences were identified between progressor B and controller C macaques. Progressor As exhibited marked neutrophilia with 3-4x more neutrophils than other groups. Both progressor groups had depleted naïve B-cell populations and controller C macaques had a greater number of CD8+ T-cells. When compared to uninfected controls, platelets and neutrophils exhibited the greatest number of significant differentially expressed genes (DEGs) were, and neutrophils had the greatest average log-fold change in gene expression. When compared to both Progressor B and Controller C macaques, Progressor As also had the greatest number of significant DEGs in platelets, but the subpopulations displaying the greatest average log-fold change in gene expression were effector CD8+ T-cells. These results reinforce evidence that disease progression occurs in multiple distinct phenotypes, as Progressor A and Progressor B macaques show highly disparate immune profiles. The greatest amount of dysregulation in progressors was observed in innate immune cells, and more CD8+ T-cells were observed in controller macaques. This supports the hypothesis that Chagas cardiac disease progression may be a function of innate immune dysregulation, and that successful control of disease depends on a stronger adaptive immune response rather than a sustained innate immune response.

6011

ALPHA-GAL VLP IMMUNOTHERAPY COMBINED WITH MILTEFOSINE OVERCOMES DRUG RESISTANCE IN VISCERAL LEISHMANIASIS

Godspower Okeke, Alexandre F. Marques

University of Southern Mississippi, Hattiesburg, MS, United States

Visceral leishmaniasis (VL), caused by Leishmania donovani, kills 20,000-30,000 people annually with no approved human vaccine. Miltefosine, the only oral antileishmanial drug, shows relapse rates of 15-31% in recent clinical data, creating an urgent treatment gap in endemic regions. Leishmania parasites express alpha-Gal epitopes on their surface; humans naturally produce anti-alpha-Gal antibodies (~1% of total IgG), a pre-existing immune axis that can be exploited therapeutically. We hypothesize that alpha-Gal Virus-Like Particle (VLP) immunotherapy using Qbeta bacteriophage, combined with miltefosine, will synergistically clear L. donovani infection and generate durable protective immunity in alpha-Gal knockout (KO) mice. Prior work from our group demonstrates that alpha-Gal VLP immunization significantly reduces lesion development in alpha-Gal KO mice challenged with L. braziliensis and L. amazonensis ($p<0.01$ at 16 weeks), and confers 100% survival against *Trypanosoma cruzi* cardiac infection versus ~60% in controls, establishing the platform across kinetoplastid parasites. Building on this, we are conducting a L. donovani VL study using four groups of alpha-Gal KO mice (C57BL/6): naive PBS control, miltefosine alone, alpha-Gal VLP immunization alone, and alpha-Gal VLP combined with miltefosine. Two groups have completed intranasal immunization (prime-boost protocol) and L. donovani infection begins March 2026. Outcome measures include parasite burden by qPCR and limiting dilution in spleen, liver and bone marrow; immune profiling by flow cytometry (Th1/Th2, M1/M2 macrophages, NKT cells) and Luminex multiplex cytokine analysis; tissue pathology by H&E and Masson trichrome histology; and anti-alpha-Gal IgG/IgA by ELISA. Data collection is anticipated by June 2026, with full results available for presentation at TropMed26. This work aims to establish a non-invasive immunochemotherapy approach that uses pre-existing human anti-alpha-Gal immunity to overcome miltefosine resistance in VL.

6012

NORTH AMERICA *TRYPANOSOMA CRUZI* TCI (GAINESVILLE) WHOLE GENOME SEQUENCING REVEALS POTENTIAL DIVERGENCE FROM DM28C REFERENCE STRAIN

Norman L. Beatty[1], Jacob Ricci[2], Massimiliano S. Tagliamonte[3], Emil Furat[3], Katelyn A. Bruno[4], Ruby E. Harrison[5], Ronald D. Etheridge[5], Carla Nartuhi Mavian[6]

[1]University of Florida, Division of Infectious Diseases and Global Medicine, Gainesville, FL, United States, [2]University of Florida College of Medicine, Gainesville, FL, United States, [3]University of Florida, Interdisciplinary Center for Biotechnology Research, Gainesville, FL, United States, [4]University of Florida College of Medicine, Department of Cardiovascular Medicine, Gainesville, FL, United States, [5]University of Georgia, Department of Cellular Biology, Athens, GA, United States, [6]University of Florida, Department of Pathology, Immunology and Laboratory Science, Gainesville, FL, United States

Trypanososma cruzi is a kinetoplastid protozoan exhibiting important features of genome expansion largely driven by multigene families encoding for GPI-anchored proteins (trans-sialidases, mucins, mucin-associated surface proteins, disperse gene family 1, GP63, and retrotransposon hot spot proteins). In the state of Florida, ongoing work has shown sylvatic and peridomestic *T. cruzi* cycles are significant in some regions, raising concerns for possible transmission to humans and companion animals. At the home of a resident bitten by triatomines, we isolated and cultured *T. cruzi* from an infected insect using a liver infusion tryptose media protocol to establish a monoculture. Using a multiplex PCR analysis this parasite was classified as discrete typing unit I (TcI). Utilizing a long-read DNA extraction method we isolated and quantified genomic DNA collected from the epimastigote monoculture. We then assembled a 37.95 Mb genome using long-read oxford nanopore technologies sequencing, generating 385 Mb of data corresponding to ~10x coverage. Our draft assembly consists of 56 contigs with a contig N50 of 912,331 and a maximum contig length of 2,006,313. Of these contigs, 6 were found to be complete based on the identification of telomeric sequences, and BUSCO analysis recovered 96.7% complete single copy orthologs across all contigs. Mapping of high confidence contigs revealed 84% alignment to recently published data for *T. cruzi* Dm28c, with a mean alignment identity of 74%. Our preliminary results reveal a potentially significant divergence from the Dm28c reference genome. Confirmation of these findings is currently being done with further assembly and annotation. Our team aims to provide the first North American *T. cruzi* telomere-to-telomere genome to improve our understanding of this parasite in the state of Florida.

6013

USE OF HYBRID CAPTURE IN NEXT GENERATION WHOLE GENOME SEQUENCING OF *LEISHMANIA MEXICANA* IN CLINICAL SAMPLES

Khalil J. Deveaux[1], J. Robert Gillespie[1], Elizabeth Dau[1], Aisha Mushtaq[1], Jiem R. Ronas[1], Frederick Buckner[1], Joshua A. Lieberman[2]

[1]University of Washington, Seattle, WA, United States, [2]University of Washington, School of Medicine, Seattle, WA, United States

Leishmaniasis is a vector-borne parasitic infection with nearly 1 million global cases reported annually. It is endemic in the United States, with locally acquired *Leishmania mexicana* species complex infections documented in Texas, Oklahoma, and Arizona. Proposed multilocus sequencing typing (MLST) schemes distinguish autochthonous *Leishmania* infections from closely related strains acquired in Central America. We aim to elucidate *L. mexicana* molecular epidemiology by performing whole genome sequencing of 34 remnant clinical samples submitted from 14 states including 5 from TX and banked by the clinical Molecular Microbiology reference lab, along with MNYL/BZ/62/M379, a previously un-sequenced culture isolate. We adapted an inexpensive hybrid capture next-generation sequencing (NGS) method developed for bacteria, "genome capture sequencing", to *Leishmania.* Extracted gDNA from

cultured *Leishmania* parasites were used to generate hybrid capture probes for NGS sequencing. A custom duplex digital PCR assay was used to quantify *Leishmania* and human abundance in each sample. NGS libraries were prepared, and hybrid-capture was performed using custom probes. The post-capture libraries were pooled, then sequenced. Of the 34 *L. mexicana* samples, we've sequenced 17. Library inputs varied from 15 to 260,000 genomes per library. Five genomes were assembled with > 98% coverage and median depth of >30. Eleven samples had >84% genome coverage, 5 with median read depths of >30. MLST analysis was completed for 15 samples, requiring >300 *Leishmania* genomes per library. MLST analysis demonstrated 12 samples and the culture isolate cladded with a Central American reference genome. Two samples formed a clade with TX3, an *L. mexicana* strain transmitted in Texas. Whole genome hybrid capture sequencing using custom probes generated high-quality genomes, even from FFPE and low parasite burden clinical samples. Preliminary phylogenetic analyses have identified 2 potential autochthonous *L. mexicana* cases. Sequencing is underway for the remaining *L. mexicana* samples, and work is ongoing to complete a whole genome phylogenetic analysis pipeline.

6014

THE EFFECTS OF ASYMPTOMATIC *PLASMODIUM FALCIPARUM* INFECTIONS, THEIR TREATMENT, AND INFLAMMATORY CYTOKINES ON COGNITIVE PERFORMANCE IN KENYAN SCHOOL CHILDREN: A PROSPECTIVE COHORT STUDY

Megan S. McHenry[1], George Ayodo[2], Irene Kagwiria Marete[3], Veronicah Knight[4], Kristen B. Cunningham[1], Erik L. Gaskin[5], Grace White[1], Wanzhu Tu[6], Chandy C. John[1], **Tuan M. Tran**[5]

[1]Ryan White Center for Pediatric Infectious Disease and Global Health, Department of Pediatrics, Indiana University School of Medicine, Indianapolis, IN, United States, [2]Center for Community Health and Wellbeing, Jaramogi Oginga Odinga University of Science and Technology, Bondo, Kenya, [3]Department of Mental Health & Behavioural Sciences, Moi University School of Medicine, Eldoret, Kenya, [4]Academic Model Providing Access to Healthcare (AMPATH), Eldoret, Kenya, [5]Division of Infectious Diseases, Department of Medicine, Indiana University School of Medicine, Indianapolis, IN, United States, [6]Department of Biostatistics and Health Data Science, Indiana University School of Medicine, Indianapolis, IN, United States

Asymptomatic *Plasmodium falciparum* (*Pf*) infections can adversely affect cognition in schoolchildren residing in malaria-endemic settings. Despite this increasing recognition, neurocognitive outcomes of asymptomatic schoolchildren carrying *Pf* blood-stage infections and their treatment have not been thoroughly assessed at the participant level. To address this gap, we performed a prospective cohort study in which Kenyan children aged 7-9 years (n=254) underwent cognitive assessments and *Pf* detection by blood smear at baseline, 4 weeks, and 8 weeks. Smear-positive children were treated with dihydroartemisinin/piperaquine (DP). Hemoglobin, *Pf* parasitemia by quantitative PCR (qPCR), and 21 plasma cytokines were also measured longitudinally. A composite cognitive score was derived from six cognitive domain scores. Linear mixed models were used to determine if *Pf* infection and treatment with DP correlated with changes in cognitive scores over time after adjusting for age, maternal education, nutritional status, anemia, and other confounders. Plasma cytokines were measured longitudinally to explore inflammatory biomarkers as correlates of parasitemia and cognition. At baseline, 100 (39.4%) children had asymptomatic *Pf* infections, of which 23 were submicroscopic. In the pre-specified analysis, qPCR-detectable parasitaemia correlated with decreased composite scores over time (adjusted β for interaction = -0.11, 95% CI:-0.19 – -0.031), whereas treatment of smear-positive infections correlated with increased composite scores over time (adjusted β for interaction=0.082, 95% CI 0.0027–0.17). The inflammatory cytokine CXCL11 was independently associated with lower cognitive scores when added to the pre-specified model (adjusted β = -0.30, 95% CI: -0.60 – -0.022), which was validated by multiple linear regression in an independent cross-sectional cohort of Kenyan schoolchildren (n=240, adjusted β = -0.18, 95% CI: -0.34 – -0.019). In summary, asymptomatic *Pf* infections were associated with decreased cognitive gains over a two-month period in young schoolchildren living in an area of intense malaria transmission.

6015

EPIDEMIOLOGY OF BLACKWATER FEVER IN CHILDREN WITH SEVERE MALARIA IN UGANDA- CLINICAL RISK FACTORS, OUTCOMES, AND RECURRENCE

Ruth Namazzi[1], Caroline Kazinga[2], Anthony Batte[3], Quique Bassat[4], Kagan A. Mellencamp[5], Chandy C. John[5], Andrea L. Conroy[5]

[1]Department of Pediatrics and ChildHealth,Makerere University College of Health Sciences, Kampala, Uganda, [2]Global Health Uganda, Kampala, Uganda, [3]Child Health and Development Center, Makerere University College of Health Sciences, Kampala, Uganda, [4]ISGlobal, Barcelona, Spain, [5]Ryan White Center for Pediatric Infectious Disease and Global Health, Indianapolis, IN, United States

Blackwater fever (BWF) has re-emerged in recent years, increasingly affecting children in Eastern Africa, but its etiology remains unclear. Historically, BWF affected primarily adults and was characterized by intravascular hemolysis, dark urine, jaundice, and acute kidney injury, and was associated with high inpatient mortality. Data on risk factors and outcomes in children are limited.We evaluated the frequency, clinical risk factors, and outcomes of BWF among 750 children hospitalized for malaria at three sites in central, eastern, and northern Uganda. BWF was defined based on parental report of tea-colored urine. The mean age of children was 5.6 years (standard deviation, 3.5) and 59.1% of participants were male. At enrolment, 440 (58.7%) children presented with BWF. Compared to children without BWF on admission, children with BWF were older (mean age, 6.2 vs. 4.5 years, p<0.001), more likely to report prior blood transfusion (39.1% vs. 11.0%, p<0.001) and prior dark urine (51.6% vs. 11.0%, p<0.001), and more likely to have jaundice, severe anemia, and acute kidney injury (P<0.001 for all), but less likely to have prostration, seizures, or coma (P<0.001 for all). Compared to children without BWF on admission, children with BWF had no increase in acute mortality associated with BWF (2.5% vs. 3.9%,p=0.284), but were hospitalized longer (mean duration in days, 4.8 vs. 3.9, p=0.003) and were more likely to require a blood transfusion (69.5% vs. 34.0%, p<0.001) and dialysis (5.2% vs. 1.9%, p=0.02). By 4-month follow-up, 58/427 children with BWF (13.6%) had a recurrent inpatient BWF event, compared to 5/298 in children without BWF during their initial admission (1.7%, p<0.001). BWF on admission was associated with an adjusted incident rate ratio of BWF of 7.07 (95% CI, 3.03 to 16.53, p<0.001) within 4 months. BWF during an episode of severe malaria is not associated with increased acute mortality but is strongly associated with prolonged hospitalization, requirement for blood transfusion and dialysis and substantial post-discharge morbidity, including increased risk of post-discharge BWF

6016

SEVERE MALARIAL ANEMIA AND MALARIA WITH COMPLICATED SEIZURES ARE ASSOCIATED WITH LOWER LONG-TERM EXECUTIVE FUNCTION SCORES IN UGANDAN CHILDREN

Kagan A. Mellencamp[1], Ruth Namazzi[2], Andrea Conroy[3], Dibyadyuti Datta[1], Robert Opika Opoka[4], Paul Bangirana[2], Chandy C. John[5]

[1]Indiana University School of Medicine, Indianapolis, IN, United States, [2]Makerere University, Kampala, Uganda, [3]Indiana University, Indianapolis, IN, United States, [4]College of Health Sciences, Makerere Univ, Kampala, Uganda, [5]Indiana University School of Medicine, Saint Paul, MN, United States

Long-term behavioral problems have been reported following cerebral malaria (CM) and severe malarial anemia (SMA), but behavioral problems in other forms of severe malaria and executive function in any form of severe malaria are not well characterized. We enrolled a cohort of 600 children 6 months to 4 years of age in central and eastern Uganda in a study of

severe malaria from 2014-2018. At 12-month follow-up, surviving children with CM (n=39), SMA (n=124), respiratory distress (RD, n=65), malaria with complicated seizures (MS, n=119), or prostration (PR, n=51) were evaluated for behavior and executive function using the Child Behavior Checklist and Behavior Rating Inventory of Executive Function-Preschool, respectively. Age-adjusted z-scores were calculated using scores from community children (CC) enrolled at the same time (n=96). Higher scores indicated worse behavioral or executive function outcomes. In linear regressions adjusted for age, sex, site, nutritional status, socioeconomic factors, HIV status, and malaria over follow-up, significant differences in internalizing behavior and global executive function compared with CC were identified in children with SMA (0.45 [0.10, 0.81] and 0.49 [0.15, 0.83]) or MS (0.44 [0.08, 0.79] and 0.59 [0.25, 0.93]), but not in children with CM, RD, or PR. After Bonferroni correction, global executive function remained significantly different between children with SMA or MS and CC. Externalizing behavior at 12 months was similar in children with severe malaria and CC. In Ugandan children younger than 5 years, SMA and MS are associated with worse long-term executive function scores. Ongoing analyses are evaluating baseline clinical and laboratory correlates of outcomes across forms of severe malaria.

6017

COGNITIVE IMPAIRMENT POST NON-SEVERE MALARIA IN ADULTS: A PROSPECTIVE COHORT STUDY IN INDIA

Vani H. Chalageri[1], Shrinivasa B. Marinaik[1], Praveen K. Tripathi[2], Himani Kashyap[3], Animesh Jain[4], Sujith Nath[5], Gunjan Negi[1], Prerana Nandish[1], Shankar G[1], Viprasha Tomer[1], Kajal Bankoti[1], Anupkumar Anvikar[6]

[1]ICMR NIMR (National Institute of Malaria Research), Bengaluru, India, [2]ICMR NIMR (National Institute of Malaria Research), Ranchi, India, [3]NIMHANS (National institute of mental health and Neuro Sciences), Bengaluru, India, [4]Kasturba Medical College, Mangalore - 575 001, Karnataka, INDIA, India, [5]ICMR Vector Control Research Centre, Puducherry, India, [6]National Institute of Malaria Research, New Delhi, India

In the clinical malaria spectrum, while cerebral malaria's neurological sequelae are well documented, long-term cognitive deficit following non-severe malaria remains underexplored, particularly among adults. This study evaluated cognitive and executive outcome following *Plasmodium falciparum* and *Pvivax* infections in adults from 2 endemic regions of India. In this prospective cohort study, adults aged 18–60 years with febrile illness were screened (n=1,196): among them, 88 eligible participants were enrolled and followed up for 8 months post-infection (42 malaria-exposed; 46 malaria-unexposed cohorts). Individuals with prior neurological/psychiatric disorders, cerebral malaria, or alcohol dependence were excluded. Following screening with the Mental State Examination/ Hindi Mental State Examination (MSE/HMSE), cognitive and executive functions were assessed at baseline(T0), followed up at 2ndmonth(T2) and 8thmonth(T8) using the Montreal Cognitive Assessment(MoCA) and Frontal Assessment Battery(FAB). Malaria-exposed cohort demonstrated significantly lower MoCA scores compared to malaria-unexposed cohort across all time points (T0;T2 $p \leq 0.001$; T8 $p=0.029$), with increasing relative risk(RR) persisting at 8th month ($RR_{T0}=1.42$; $RR_{T2}=1.75$; $RR_{T8}=1.74$). Executive dysfunction in malaria-exposed cohort was observed only at baseline (FABscore $p \leq 0.001$) but showed temporal improvement. Repeated-measures analysis demonstrated lower overall cognitive performance in the malaria-exposed cohort($\eta^2=0.112$), with improvement over time($\eta^2=0.185$) and no significant group–time interaction. These findings indicate that non-severe malaria infection in adults is associated with persistent cognitive impairment extending several months beyond acute infection, challenging its perception as fully reversible and underscoring the need to consider broader morbidity outcomes in malaria control program. Incorporating standardised cognitive screening and structured follow-up into routine malaria case management may enable early detection of cognitive deficits to improve long term patient outcome.

6018

MENTORSHIP IDENTIFIES GAPS IN CLINICAL MANAGEMENT AND STRENGTHENS HEALTH WORKER CAPACITY IN SEVERE MALARIA MANAGEMENT IN WESTERN KENYA

Elvis Oyugi[1], Agatha Mandu[2], Seraphine Nthenge[1], Beth Barasa[3], Alphonce Nyakech[3], Dan Abaja[1], Sammy Mahugu[4], Donald Apat[5]

[1]PATH, Kisumu, Kenya, [2]PATH, Kakamega, Kenya, [3]PATH, Homa Bay, Kenya, [4]National Malaria Control Program, Ministry of Health, Nairobi, Kenya, [5]PATH, Nairobi, Kenya

Kenya accounts for 1.5% of global malaria cases and deaths. Gaps in clinicians' knowledge and skills in severe malaria management persist in high-malaria-burden region of Western Kenya despite availability of national malaria diagnosis and treatment guidelines. REACH Malaria project implemented hands-on mentorship for frontline healthcare workers and evaluated clinicians' competency to manage severe malaria cases, adherence to national treatment guidelines and patient outcomes. We analyzed routine severe malaria data and purposively selected health facilities with sub-optimal (<80%) inpatient reporting rates, for mentorship. In July and November 2025, clinical mentors reviewed inpatient records using a standardized checklist to assess adherence to severe malaria guidelines (triage, diagnosis, and treatment). Mentorship included bedside coaching, continuing medical education (CMEs), and webinars. Competency (≥80% score) was calculated using a composite indicator of correct triage, diagnosis, and treatment. A total of 625 clinicians from 166 facilities (17.5% of all admitting facilities in high malaria burden region of Western Kenya) were mentored. Among 1,278 inpatient records, signs and symptoms were documented in 82.5%, parasitological diagnosis in 96%, and injectable artesunate was prescribed in 86.7%, with 96.1%, 92.3%, and 87.2% receiving the first, second, and third doses, respectively. Vital signs monitoring was low (24.4%), and 12.2% of patients were not transitioned to oral artemether-lumefantrine. Injectable artesunate was available in 95.5% of facilities and correctly administered by 94% of clinicians. Overall, 83.2% of clinicians achieved competency. Reductions of 44.0% in severe malaria admissions and 61.1% in mortality attributed to severe malaria were observed in December 2025, compared to June 2025. Majority of the clinicians were competent in severe malaria management according to national treatment guidelines, though gaps exist in patient monitoring and treatment transition. We recommend structured mentorship to improve clinical capacity and quality of care in high burden settings.

6019

BEYOND THE SMEAR: MULTIPLEXED POINT-OF-CARE DIAGNOSTICS USING LABEL-FREE IMAGING CYTOMETRY

Paul Martin Lebel[1], Nicholas Bratvold[2], Ilakkiyan Jeyakumar[3], Gregory Suematsu[3], Michelle Wei Ling Khoo[4], JAMES EMORUT[5], Genay Pilarowski[6], Chaz Langelier[7]

[1]Biohub, San Francisco, CA, United States, [2]Chan Zuckerberg Biohub SF, San Francisco, CA, United States, [3]San Francisco, CA, United States, [4]CZ Biohub SF, San Francisco, CA, United States, [5]Redwood City, CA, United States, [6]Chan Zuckerberg Initiative-Biohub, San Francisco, CA, United States, [7]UCSF, San Francisco, CA, United States

Access to critical care diagnostics remains a fundamental bottleneck in global health, with nearly half of the world's population lacking access to essential tests. This gap is especially acute in syndromic conditions such as febrile illness, where distinct diseases present with overlapping symptoms but require different treatments. Existing approaches based on single-disease rapid tests, centralized laboratories, or symptom-based assessment are poorly suited to point-of-care decision-making. Building on prior malaria diagnostic work using the Remoscope imaging cytometer platform, we show that multiple clinically relevant health and disease states can be distinguished from ~10 µL of unstained peripheral blood within minutes at low cost. Millions of blood cells are flowed, imaged, and interpreted by AI models with onboard compute hardware. Two categories of custom models were developed: (1) weakly supervised classifiers trained

on patient-level labels that integrate disease-specific features across hundreds to thousands of images, and (2) semi-supervised models for cell type and state discovery. We show that in addition to malaria, the classifiers can distinguish sepsis-positive and sepsis-negative adult patients in intensive care units, offering a way to address this common and high-risk diagnostic challenge. We also demonstrate separation of white blood cell types and activation states, and red blood cell abnormalities such as malaria infection, poikilocytosis, anisocytosis, and nucleation, suggesting further applications in hemoglobinopathies such as sickle cell disease. Finally, we demonstrate proof of concept on the cellular assessment of cerebrospinal fluid (CSF) for diagnosis of neurological conditions on the same platform. Together, these findings support the potential of a single AI-enabled platform to deliver multiplexed diagnostic information at the point of care, expanding access to critical diagnostics in resource-limited settings.

6020

CLINICAL CHARACTERISTICS, AETIOLOGY AND COMORBIDITIES OF CENTRAL NERVOUS SYSTEM INFECTION DEATHS AMONGST CHILDREN UNDER-FIVE YEARS IN WESTERN KENYA

Florence Achieng' Aweyo

Kenya Medical Research Institute, Kisumu, Kenya

Central Nervous System (CNS) infections are major contributors to under-five morbidity, mortality, and neurological complications globally, disproportionately in sub-Saharan Africa. In Western Kenya, CNS infections are frequently identified in mortality surveillance, but gaps persist in understanding their clinical presentation, diagnostic pathways, and etiologic profiles. We conducted data analysis of post-mortem confirmed CNS infection deaths enrolled in the Child Health and Mortality Prevention Surveillance (CHAMPS) program in Western Kenya between May 2017 and December 2025. Causes of death (COD) were determined by a panel of experts using data from post-mortem examinations conducted through minimally invasive tissue specimen testing, clinical records, and verbal autopsy. We described clinical features, antemortem diagnoses, pathogen distribution, and comorbidities. Among 1467 deaths, CNS infections accounted for 10.9% (160). Cerebral Malaria was most prevalent (134, 83.8%), followed by meningitis (16, 10.0%), and encephalitis (13, 8.1%). Of meningoencephalitis cases, only 15.7% were clinically diagnosed and lumbar puncture done in 10.5 % antemortem, yet 74% presented with suggestive symptoms like convulsions. Leading pathogens included *Plasmodium falciparum* (72.5%), *Klebsiella pneumoniae* (9.2%), and *Streptococcus pneumoniae* (6.5%). Pathogen distribution varied by HIV exposure, nutrition status, age group and prematurity. Common comorbidities included pneumonia (42.0%), malnutrition (40.5%), sepsis (28.2%), and severe anemia (23.7%). Polymicrobial infections were reported. CNS infections contribute substantially to under-five mortality in Western Kenya although markedly underdiagnosed before death. Malaria remains the dominant etiology, with important contributions from bacterial pathogens and comorbidities. Strengthening early recognition, diagnostic capacity (including lumbar puncture), and integrated management of comorbidities is critical to reducing preventable deaths.

6021

MULTIPLEX SEROSURVEILLANCE REVEALS CROSS-PATHOGEN VULNERABILITIES IN SOUTH SUDAN

Sophie Berube[1], Sarah Lapidus[2], Stephen Ohidor[3], Alyssa Sbarra[4], Amy Wesolowski[2], Tania Gonzalez[5], Nicholas A. Presley[6], Lochebe Boniface[3], Yak Yak Bol[7], Angelia Sanders[5], Kelly Callahan[5], Saki Takahashi[4], Sonia Hegde[2], Scott Nash[5]

[1]University of Florida, Gainesville, FL, United States, [2]Johns Hopkins University, Baltimore, MD, United States, [3]The Carter Center, Juba, South Sudan, [4]Baltimore, MD, United States, [5]Atlanta, GA, United States, [6]Carter Center, Atlanta, GA, United States, [7]Ministry of Health

Past pathogen exposure can be measured by quantifying antibody levels to antigens. Multiplex serological assays simultaneously quantify dozens of antigen-specific antibodies, and, therefore, measure exposure to multiple pathogens furnishing insights into co-occurring pathogen exposures in individuals and populations. Using IgG serological responses to 33 antigens from neglected tropical diseases (NTDs), malaria, and vaccine-preventable diseases (VPD)s from population-based trachoma surveys (89 clusters; N=9,603 individuals) in South Sudan (2024), we examined cross-pathogen vulnerabilities in individuals and across space. We identified individual-level associations between past exposure to different antigens using generalized linear and mixed effect models. To holistically assess vulnerability to infectious diseases, we calculated cluster-based vulnerability scores by combining relative the seroprevalence of all antigens within a cluster using a Bayesian approach. We then correlated cluster-level seroprevalence of specific markers with overall vulnerability. Within individuals, several significant positive associations between seropositivity to a range of vector-borne diseases like dengue and zika (OR range: 1.5-1.7, $p<0.05$), indicate co-occurrence of vector-borne diseases. The vulnerability scores exhibited high spatial heterogeneity within the study area. Furthermore, higher cluster-level vulnerability scores were significantly correlated (Spearman's Rho range: 0.4-0.74) with increased seroprevalence to at least one antigen from 9/10 NTDs measured in the cohort but none of the VPD antigens. This result suggests a role for NTDs as sentinel markers of high overall infectious pathogen burden and potential vulnerability to infectious disease. These findings highlight opportunities to target interventions that simultaneously address multiple pathogens, such as vector control, at fine spatial scales.

6022

HYPERKALEMIA IS ASSOCIATED WITH INCREASED MORTALITY AND NEUROLOGIC DEFICITS IN CHILDREN WITH SEVERE MALARIA IN UGANDA

Michael D. Lintner Rivera[1], Violah Nakulima[2], Anthony Batte[3], Caroline Kazinga[2], Quique Bassat[4], Ruth Namazzi[3], Chandy C. John[1], Andrea Conroy[1]

[1]Ryan White Center for Pediatric Infectious Disease and Global Health, Indiana University School of Medicine, Indianapolis, IN, United States, [2]Global Health Uganda, Kampala, Uganda, [3]Makerere University, Kampala, Uganda, [4]ISGlobal, Barcelona, Spain

Severe malaria is a leading cause of pediatric mortality and is increasingly recognized as a syndrome of multi-organ dysfunction, including acute kidney injury (AKI), metabolic acidosis, and hemolysis. Hyperkalemia is a life-threatening electrolyte disturbance in critical illness, but its burden and clinical implications in pediatric severe malaria remain incompletely characterized. We evaluated the prevalence, risk factors, and outcomes of hyperkalemia in a prospective cohort of 748 children aged 3 months to <16 years hospitalized with severe malaria across three Ugandan sites. The mean age of participants was 5.5 years (standard deviation, 3.5), and 59.1% were male. Hyperkalemia (potassium >5.5 mmol/L) was present in 8.2% of children at enrollment and occurred in 12.8% during hospitalization. Hyperkalemia was strongly associated with AKI, with markedly higher serum creatinine levels in affected children (median 4.6 vs 0.4 mg/dL, $p<0.0001$). Independent clinical predictors of hyperkalemia included a history of low urine output, blackwater fever, or hypertension on admission ($p<0.05$ for all). Hyperkalemia refractory to medical management was an indication for dialysis in 10/29 (34.5%) children who received dialysis. Children with hyperkalemia had higher in-hospital mortality compared to those without (10.5% vs 2.0%, $p<0.001$), longer hospital stays (median of 7 days vs. 3 days, $p<0.001$), and increased neurologic deficit at discharge (10.6% vs. 5.2%, $p=0.049$). Hyperkalemia was also associated with kidney disease at one-month follow-up (48.8% vs. 21.4%, $p<0.001$) and progression to chronic kidney disease by 4-months (14.1% vs. 4.0%, $p<0.001$). These findings demonstrate that hyperkalemia in pediatric severe malaria identifies a high-risk phenotype associated with kidney dysfunction and adverse outcomes. Early identification of hyperkalemia using point-of-care testing may provide a practical and scalable approach to risk stratification for critically ill children with severe malaria in resource-limited settings.

6023

PRAGMATIC EVALUATION OF TREATMENT PLAN HANDOFFS AT A TELEMEDICINE AND MEDICATION DELIVERY SERVICE IN HAITI: A PROSPECTIVE QUANTITATIVE STUDY

Molly Klarman[1], Youseline Cajusma[1], Jude Ronald Beausejour[2], Lerby Exantus[3], Madsen Beau De Rochars[1], Chantale Baril[3], Eric Jorge Nelson[1]

[1]University of Florida, Gainesville, FL, United States, [2]Independent clinical practice and affiliation, Les Cayes, Haiti, [3]Université d'État d'Haiti- Faculté de Médecine et de Pharmacie, Port au Prince, Haiti

Medication adherence is critical to achieving positive clinical outcomes in healthcare. Adherence depends in part on effective health communication at the time medications are provided. While telemedicine can reduce barriers in accessing healthcare, virtual-care is more vulnerable to communication failures, compared to traditional in-person care. To characterize this vulnerability, we evaluated treatment plan handoffs from providers to families at a telemedicine and medication delivery service (TMDS) called MotoMeds in Haiti. We used an evaluation framework encompassing two components of medication adherence: initiation and implementation. Families were interviewed by research staff 12-48 hours after engagement with the TMDS. Outcomes included treatment initiation and comprehension of plan implementation, assessed through structured surveys and medication demonstrations. A total of 258 caregivers were enrolled; 94% were female, 87% were parents, and 37% had not completed primary school. Among the three most common treatment plans evaluated, medication initiation rates were 73% (150/206) for dehydration (oral rehydration solution [ORS]), 91% (127/140) for fever/pain (liquid paracetamol), and 94% (130/139) for suspected systemic bacterial infections with oral antibiotics indicated. Non-initiation events were most often medical condition related (59%, 44/74), and the TMDS failed to provide medication/ supplies in 7% (5/74) of events. Correct recall of all implementation steps was 73% (150/206) for dehydration, 96% (123/140) for fever/pain, and 76% (106/139) for suspected systemic bacterial infections. Common implementation errors included incorrect ORS mixing ratios, underestimation of paracetamol dosing frequency, and overestimation of antibiotic duration. Our findings suggest most caregivers initiated treatment and received appropriate medications/supplies, however, meaningful and actionable gaps in implementation remain. Targeted initiatives to improve communication are needed to ensure the TMDS model supports the realization of intended health outcomes.

6024

EVALUATING A GAMIFIED PSYCHOLOGICAL INOCULATION INTERVENTION TO ADDRESS VACCINE HESITANCY: A MIXED METHODS STUDY

Zeeshan Ahsan[1], Raheel Allana[1], Nazia Ahsan[1], John Cook[2], Zoyia Hasan[1], Areeba Ahmer[1], Wendy Cook[3], Chelsey Lepage[4], Kathryn L. Hopkins[5], **Abdul Momin Kazi**[1]

[1]Aga Khan University, Karachi, Pakistan, [2]University of Melbourne, Melbourne, Australia, [3]Stanford University School of Medicine, California, AB, United States, [4]Irimi Company, Canada, CA, Canada, [5]National Institute for Health and Care Excellence, University of Cambridge, United Kingdom

Vaccine hesitancy continues to hinder childhood immunization efforts in Pakistan. Psychological inoculation preemptively builds resistance to misinformation by exposing individuals to weakened forms of misleading arguments offers a promising digital intervention. This study evaluates the cultural adaptation and effectiveness of the Cranky Uncle Vaccine game in the Pakistani context. A two-phase mixed methods design was employed. In Phase 1, co-design workshops (N = 60) in Karachi informed the cultural adaptation of game characters and narratives. In Phase 2, a pre-test/post test pilot study (N = 1,265) was conducted in urban and semi-rural settings (Karachi and Matiari). Participants engaged with Urdu and Roman Urdu versions of the game. Changes in vaccine attitudes, perceived importance, intentions, and misinformation detection were assessed using paired t tests and linear regression analyses. Co-design workshops produced culturally resonant adaptations, including transforming the "Cranky Uncle" into "Khabti Chacha," a relatable rural archetype, and redesigning the nurse character to reflect Sindhi norms of modesty and professionalism. Participants highlighted the game's effectiveness in modeling respectful dialogue, while recommending reduced text density for low-literacy users. In the pilot phase, the Urdu-speaking cohort demonstrated significant improvements in vaccine attitudes ($p = .02$), perceived importance ($p = .01$), and vaccination intentions ($p = .01$). Although overall effect sizes were small ($d = 0.09$-0.10), subgroup analyses revealed stronger effects among vaccine hesitant individuals. Regression results indicated that lower baseline acceptance predicted greater gains in factual beliefs and vaccination intent. Notably, 88.5% of hesitant participants shifted to being likely to vaccinate after gameplay. The culturally adapted Cranky Uncle Vaccine game is a feasible and effective intervention for enhancing resistance to misinformation in Pakistan. Findings highlight the potential of gamified psychological inoculation among vaccine-hesitant populations.

6025

COMMUNITY QUALITY IMPROVEMENT TEAMS IMPROVE ANTENATAL CARE UPTAKE AND QUALITY IN WESTERN KENYA: RESULTS OF A PRAGMATIC CLUSTER-RANDOMISED TRIAL

Gerald Ong'ayo[1], Hellen Cherono Barsosio[2], James Dodd[3], Antony Ochung[4], Tansy Edwards[3], Lilian Otiso[5], Vicki Doyle[6], Gordon Okomo[7], Tilly Fox[3], Feiko ter Kuile[3], Miriam Taegtmeyer[3], C-it-DU-it Consortium[8]

[1]KEMRI-CGHR, Kisumu, Kenya, [2]Kenya Medical Research Institute, Kisumu, Kenya, [3]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [4]Kenya Medical Research Institute - Centre for Global Health Research, Kisumu, Kenya, [5]LVCT Health, Nairobi, Kenya, [6]Capacity Development International, Liverpool, United Kingdom, [7]Homabay County Department of Health, Homabay, Kenya, [8]Kenya

Less than 10% of pregnant women in Sub Saharan Africa achieve WHO's recommended 8 antenatal care (ANC) contacts for optimal pregnancy management. Continuous Quality Improvement (QI) approaches have been used at facility level in low-middle-income countries but have not previously been evaluated in a randomised trial context, especially integrated community-and-facility QI teams. QI teams use routine data to identify challenges, develop context-specific solutions and measure progress towards addressing them. We leveraged digital linkage of routine community and facility electronic patient data for ANC (control arm) to assess the impact of integrated community-and-facility QI teams using these linked data on ANC uptake and services (intervention arm), in Western Kenya. This was a pragmatic cluster randomised trial with 18 health facilities (clusters) randomly allocated (1:1) to control or intervention arms. QI teams comprised multi-disciplinary representatives from the target population, health facility and community health teams. Our primary outcome was uptake of 8 ANC contacts determined at delivery and/or 6 weeks postnatal follow-up. We used a binomial multilevel (mixed effects) regression model with facility (cluster) as random effect. Risk ratios and confidence intervals were determined using bootstrapping and p-values estimated using parametric bootstrap procedures. Between Feb 2025 and Feb 2026, 1454 women were recruited and 1444 (99.0%) followed up. Demographic characteristics were similar in each arm, with median age 24 (IQR 20-29) years, largely married (77%), having completed secondary school education (48%), and with 2-3 prior pregnancies (41%). Preliminary analysis showed a strong benefit of QI teams on uptake of 8 ANC contacts - 325/712 (45.6%) versus 112/732 (16.3%) in the control arm (adjusted risk ratio 2.59 (95% CI 1.88 - 3.42, $p<0.001$). Our approach led to an important increase in ANC uptake. Attaining the recommended 8 ANC contacts is a critical first step in improving maternal health outcomes, and our findings are of comparable importance to trials reporting significant prenatal diagnostics/treatments.

6026

COMMUNITY-BASED HPV SCREENING FOR FEMALE SEX WORKERS IN ACCRA: CHALLENGES IN RECRUITMENT AND RETENTION IN A LARGE HPV STUDY

Mary Duffie Osei[1], Frank E. Baiden[2]

[1]Research and Development Division, Ghana Health Service, Accra, Ghana, [2]Fred Newton Binka School of Public Health, University of Health and Allied Sciences, Hohoe, Ghana

Female sex workers (FSWs) are at increased risk of human papillomavirus (HPV) infection and cervical cancer, yet screening uptake in this population remains low in Ghana. The large HPV study is a longitudinal five-phase cohort study to determine the incidence and patterns of HPV infections among 500 FSWs in Accra. This study explored experiences in the recruitment and study retention (RSR) of participants. Semi-structured interviews (SSIs) were conducted with field coordinators at the five study hubs. These were followed by in-depth interviews with lead officers. The interviews explored behavioral, social, and logistical issues related to RSR. Data was analyzed using thematic analysis with MAXQDA software, inductive and deductive coding techniques were also used to identify emergent themes. Notes made by the field teams were reviewed to improve understanding of the process involved in assuring successful RSR. Fifteen field coordinators and four team leads participated in the SSIs and IDIs respectively. Participants indicated that, although collaboration with a local NGO (the Ghana-West Africa Program to Combat AIDS and STI) effectively facilitated recruitment and trust, sustaining interest and continued participation were challenging. They identified strong gatekeeping and hierarchical systems in the ecosystems of commercial sex work as key elements that required respectful and tactful approaches. Stigma, apprehensions about law enforcement and mobility were challenges that equally required pragmatic-thinking to ensure successful RSR. Competing priorities, misconceptions about repeat testing, impersonations, and frequent changes in names and contact information were challenges the study team had to adapt to. Continuous NGO engagement through peer networks, sustained trust-building, flexibility in strategies, and cooperation with gatekeepers helped to ensure successful RSR. Adaptive strategies are needed in longitudinal research with female sex workers in Accra, Ghana. Strong partnerships with respected local NGOs and respect for hierarchical structures in the sex work ecosystem were critical to successful RSR.

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DISSEMINATION OF MALARIA TREATMENT TRIAL RESULTS TO STUDY PARTICIPANTS IN CAMBODIA, ETHIOPIA, AND PAKISTAN: A QUALITATIVE STUDY

Sarah A. Cassidy-Seyoum[1], Bipin Adhikari[2], Muthoni Mwaura[1], Asia Khan[3], Shan-e-Zehra Zaidi[3], Zahida Azizullah[3], Bushra Qurashi[3], Kansite Gellebo[4], Samuel Alemu Bamboro[5], Mom Eanst[2], Rupam Tripura[2], Hem Vattanak[2], Thy Vanda[2], Kem Sovann[2], Ou Simvieng[2], Phal Chanpheakdey[2], Hellen Mnjala[1], Grant Lee[1], Najia Ghanchi[3], Phaik Yeong Cheah[2], Ric N. Price[1], Mohammad Asim Beg[3], Tamiru Shibiru Degaga[5], Lek Dysoley[6], Kamala Thriemer[1]

[1]Global and Tropical Health Division, Menzies School of Health Research, Darwin, Australia, [2]Mahidol-Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand, [3]Department of Pathology and Laboratory Medicine, Aga Khan University, Karachi, Pakistan, [4]Department of Sociology and Social Anthropology, Arba Minch University, Arba Minch, Ethiopia, [5]College of Medicine & Health Sciences, Arba Minch University, Arba Minch, Ethiopia, [6]National Center for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia

Dissemination of study results to research participants is an ethical imperative, yet it is rarely implemented. Best practices for result dissemination, especially in low-and middle-income countries, are lacking. Prior findings from a multi-site clinical trial of *Plasmodium vivax* treatment options showed that participants' preferences for receiving results varied across settings. These preferred approaches were implemented in three of the four study countries. The purpose of this study was to assess and explore these dissemination activities from a participant's perspective. Attendees were invited to participate in focus group discussions (FGDs), and a subset were able and agreed to participate. There were 25 participants in Cambodia, 34 participants in Ethiopia, and 23 participants in Pakistan. Recordings were transcribed and translated, transcripts were reviewed, an inductive codebook was developed, and a thematic analysis was conducted. The dissemination activities were well accepted by participants despite an expectation of some to receive individual results rather than only aggregated trial results and suggestions for broader community dissemination. Dissemination activities were successful in transferring knowledge pertaining to the trial and its results, research in general, and malaria health literacy. Study findings suggest that dissemination could create or did not correct some misconceptions, including blurring the line between research and routine care. However, the dissemination process had positive impacts, including building trust and improving health literacy, whilst making participants feel part of a larger endeavor. Participants were satisfied with dissemination activities. They were effective in making them feel recognized and contributing to their knowledge of study results and/or malaria. Our findings underscore the importance of consulting participants about their preferences for dissemination and tailoring activities accordingly, as well as potentially providing individual results and implementing broader community dissemination.

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MPXV VACCINE ACCEPTANCE AMONG MEN WHO HAVE SEX WITH MEN, PROFESSIONAL SEX WORKERS AND OTHER AT RISK URBAN POPULATIONS IN SOUTH EASTERN DR CONGO

Sydney Merritt[1], Patrick K. Mukadi[2], Megan Halbrook[1], Michael Beya[2], Merly Tambu Yinga[2], Andrea Mauyma Claudel[2], Aurelie Yandjo Akambo[2], Yvon Anta Mongosha[2], Jean Paul Kompany[2], Christina Melissa Frederick[3], Nicole A. Hoff[1], Placide Mbala-Kingebeni[2], Jason Kindrachuk[3], Anne Walsh Rimoin[1]

[1]Jonathan and Karin Fielding School of Public Health, University of California, Los Angeles, Los Angeles, CA, United States, [2]Institut National de Recherche Biomedicale, Kinshasa, Democratic Republic of the Congo, [3]Max Rady College of Medicine, University of Manitoba, Winnipeg, MB, Canada

Monkeypox virus (MPXV) has long been endemic in the Democratic Republic of the Congo (DRC). While historically characterized by zoonotic spillover events and isolated rural clusters among children, recent identification of a new MPXV subclade has shifted the understanding of the disease. In 2024, Clade Ib was first identified among professional sex workers in Eastern DRC. Subsequently, the virus has been introduced into large urban centers such as Kinshasa and Goma, as well as neighboring countries which had not previously reported cases of mpox. Given the 2022 global expansion of the virus among men who have sex with men (MSM), identification among professional sex workers (PSW), and expanding geographic range of the virus, we sought to characterize mpox vaccine acceptance, as well as more broadly, knowledge, attitudes and practices about the disease. This survey was specifically conducted in two urban centers, Lubumbashi, Haut Katanga province, and Kolwezi, Lualaba province, where mpox had not been widely reported as of December 2025. Among 988 respondents, we enrolled 214 MSM (21.7%), 453 PSW (45.9%), 357 other at risk population members (36.1%) and 36 individuals who identified as both MSM and PSW. Stratified by population type, less than half of all respondents had heard of the disease mpox, with 41.6%, 32.9% and 49.3% of MSM, PSW and at-risk populations responding "yes", respectively. However, among these same respondents, over 70% reported that they were "somewhat concerned" or "very concerned" about mpox. Overall, mpox vaccination was widely accepted across all populations, with very positive responses from 84.8%, 67.1% and 68.9% of MSM, PSW and at-risk respondents. These results provide invaluable insights into mpox knowledge in a region where the virus had yet to circulate widely. In contrast with previous studies which had identified mpox vaccine acceptance at 61% among health care workers nationally, these results show improvement

in general vaccine acceptance. Moreover, this research has identified key gaps in public health communication, and a high tolerance for future vaccine rollout among those most at risk populations.

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INTEGRATING TELEHEALTH INTO PRE-EXPOSURE PROPHYLAXIS (PREP) IMPLEMENTATION STRATEGIES IN INDIA: A QUALITATIVE ANALYSIS OF STAKEHOLDER PERSPECTIVES

Natasha Glendening[1], Sara Piao[2], Katarina Scala[2], Katherine Lewis[3], Crystal Maxson[3], Suchith Kumar[4], Jayee Chowdhury[5], Sukanta Paul[5], Asim Sen[5], Akram Pasha[6], Bhagya Lakshmi[6], Pranathi Prakash[6], Syed Hafeez Ur Rahman[6], KT Venukumar[6], Dallas Swendeman[2], Protim Ray[5], Sushena Reza-Paul[6], Annie Fehrenbacher[3]

[1]Santa Clara University, Santa Clara, CA, United States, [2]University of California Los Angeles, Los Angeles, CA, United States, [3]University of Southern California, Los Angeles, CA, United States, [4]SK Professional Translation and Transcription Services, Mysore, India, [5]Durbar Mahila Samanwaya Committee (DMSC), Kolkata, India, [6]Ashodaya Samithi, Mysore, India

India has the second largest HIV epidemic in the world, with an estimated ~2.6 million people living with HIV. One method that could be utilized to combat HIV prevalence is the use of Pre-exposure Prophylaxis (PrEP), a medication shown to have strong efficacy to prevent HIV infections. However, whilst PrEP was approved in India in 2016, uptake in the country remains low. One mechanism for PrEP implementation that could improve PrEP uptake and adherence is the integration of Telehealth in service delivery. This qualitative study aimed to assess stakeholder perspectives on the role of Telehealth in PrEP implementation in India. In partnership with two community based organizations that have long-standing targeted intervention programming in Mysore and Kolkata, we recruited n=42 participants for in-depth interviews. These included n=20 internal stakeholders and n=22 external stakeholders, all of whom had had experience with PrEP implementation in India. Interviews took place between August 2023-February 2024 and were conducted in Kannada, Bangla, Hindi or English. Transcripts were transcribed and uploaded to Dedoose for analysis using an inductive thematic approach. We found three overarching themes including: 1) Telehealth as a tool utilized in PrEP programs; 2) Telehealth as a facilitator of PrEP implementation; 3) Telehealth as a barrier of PrEP implementation. Telehealth has been used to send medication reminders, monitor side effects, and to provide counseling. Stakeholders also viewed Telehealth as a facilitator of PrEP implementation by improving medication adherence, accessibility to hard-to-reach populations, and delivery accessibility. However, stakeholders also noted that telehealth allows for miscommunication and can be inaccessible for some people. Data security is also a concern. Overall, telehealth integration into PrEP implementation across India has the potential to facilitate greater access and adherence to HIV prevention medication according to stakeholders. However, the effectiveness of telehealth delivery is dependent on the ability to address communication, accessibility and data-security concerns.

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IMPLEMENTATION LESSONS, OPPORTUNITIES, AND IMPACT OF DIGITAL HEALTH TOOLS IN MULTI-HEALTH CAMPAIGN INTERVENTIONS IN 14 STATES IN 2025: A CASE STUDY OF MALARIA CONSORTIUM NIGERIA

Dorcas Elijah Essien[1], Jerry Rago[1], Sandra Nguwasen[2], Chinedu Joseph Okoye[1]

[1]Malaria Consortium, Abuja, Nigeria, [2]Equidhi, United Kingdom, United Kingdom

Malaria Consortium implements large-scale child health campaigns in Nigeria, including Seasonal Malaria Chemoprevention (SMC), azithromycin mass drug administration (AZM), vitamin A supplementation (VAS), and integrated SMC-routine immunization campaigns. Historically, these interventions relied on paper-based systems that limited accountability, data visibility, and timely decision-making. To address these challenges, Malaria Consortium incrementally scaled digitization from a pilot in Kebbi State during the SMC campaign to fully digitalize all health campaigns across 14 states in 2025 using the DIGIT Health Campaign Management (HCM) platform. By 2025, the platform supported more than 70,000 trained users across 14 states, registering over 16 million beneficiaries and executing 15 campaign rounds, with verified coverage of 80–95%. Unique child identifiers enabled cross-campaign tracking; in Kebbi, over 2.2 million child records were matched across AZM and SMC datasets with 95% overlap across 14 local government areas. Real-time geotagged reporting improved supervision, accountability, and evidence-based decision-making, while consolidation into a single platform reduced annual operational costs from USD 150,000 to USD 30,000. Digitalization improved campaign coordination, continuity of child-level care, transparency and data use. Key lessons highlight the importance of interoperable and scalable systems, strong government ownership, adaptive management driven by user feedback, and robust offline functionality supported by appropriate infrastructure, training, and tiered technical support. The 2025 experience shows that well-implemented digital tools can improve operational efficiency, data quality, and accountability across multi-program interventions, offering a model for national scale-up. Sustaining these gains will require continued investment in interoperability, data governance, and capacity-building across the health system.

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LEVERAGING A SPATIAL DECISION SUPPORT SYSTEM TO STRENGTHEN PRIMARY HEALTH CARE PLANNING IN MALABO DISTRICT, EQUATORIAL GUINEA

Jeremias Nzamio Mba Eyono[1], Restituto Mba Nguema[1], Teodora Alene Nfa[2], Trifonia Alberta Obono Bee[2], Juanita Andeme[2], Yusleybis Veliz Cabrales[2], Santiago Eneme Mbang[1], Marcos Mbulito Iyanga[1], Faustino Etoho Ebang[1], Jordan Smith[3], David Galick[1], Matilde Riloha Rivas[2], Wonder Philip Phiri[1], Carlos Alberto Guerra[4], Guillermo A. Garcia[3]

[1]MCD Global Health, Malabo, Equatorial Guinea, [2]National Malaria Control Program, Malabo, Equatorial Guinea, [3]MCD Global Health, Hallowell, ME, United States, [4]MCD Global Health, Quito, Ecuador

Accurate population data by age group, health condition, and vaccination status are a persistent gap in primary health care planning in Equatorial Guinea. The absence of a residential address system has historically prevented household-level health planning, particularly in Malabo district, the most densely populated district on the island. In 2025, leveraging a spatial decision support system that uniquely identifies households and provides up-to-date denominators, a health census was conducted with the aim of classifying the population into health risk groups. The goal was to survey at least 95% of the inhabited households. The work was divided into 176 surveyors were organized in 103 teams to work between October and December. A total of 74,148 households were visited, and 54,937 of the 61,962 planned households were surveyed, achieving 89% coverage and capturing a total of 202,545 individuals. The target population for vaccination (i.e. children ≤5 years old) was 23,401, 44.7% lower than the projections of the Expanded Program on Immunization, which estimated 42,317 children. In terms of risk groups, 87.5% of the population were classified into groups I and II, representing those who are presumably healthy and those exposed to risks that could affect their health, while 7.3% and 3.6% were classified into risk groups III (people with chronic diseases) and IV (people with disabilities), respectively. These findings provide the first reliable population health baseline for Malabo district to critically inform vaccination targets, chronic disease program planning, and resource allocation across the primary health care system.

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THE EFFECTS OF PROFESSIONAL VERSUS COMMUNITY-LED WATER SYSTEMS ON WATER QUALITY AND CHILD GROWTH UNDER VARYING RAINFALL CONDITIONS: AN ANALYSIS OF THE ECUADORIAN NATIONAL INFANT UNDERNUTRITION SURVEY

Gwenyth O. Lee[1], Andrea Sosa-Moreno[2], Joseph Eisenberg[3], Samuel Schlesinger[4]

[1]Rutgers, the State University of New Kersey, New Brunswick, NJ, United States, [2]University of Michigan, Ann Arbor, MI, United States, [3]Univ of Michigan School of Public Health, Ann Arbor, MI, United States, [4]Green Empowerment, Tena, Ecuador

Community-led water systems, which engage local communities in managing water delivery services, are common in small and rural communities, especially in low- and middle-income countries. These systems can be tailored to local capacities, but also carry risks of poor service quality, resulting in unsafe drinking water. In contrast, professional water systems, managed in Ecuador by local governments, are often better resourced to maintain service and water quality. Our research questions are 1) How does piped-water quality differ between community-led versus municipally managed water systems, and how might extreme rainfall events modify this relationship? and 2) How is child growth (height and weight) differentially impacted by community-led versus municipal water systems? We used the publicly available 2022-2024 *Encuesta Nacional Sobre Desnutrición Infantil* (ENDI) survey to evaluate data from rural households with children under five years old who obtain their water primarily from a piped water system. To address our first aim, we constructed logistic regression models. To address our second aim, we used inverse probability weighed regression adjustment. 22% of rural Ecuadorian children receive water from a community-led system. The odds of identifying an *E.Coli* concentrations of 100MPN/100mL or greater were 2.39 times greater in a water sample from a community-led piped water system versus a municipal water system. Greater increases in contamination following extreme rainfall were observed in samples from community-led systems compared to municipal ones, which suggests that these systems are less resilient to climatic stressors. After accounting for potential confounders, the average treatment effect of receiving piped water from a community-led versus municipally managed water system was -0.08 height for age Z-scores. In comparison, the average treatment effect of receiving water from an unpiped source, versus a municipal piped source, was -0.17 height for age Z-scores. These results suggest a need for increased support for community-led water services.

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PROGRAM EVALUATION OF THE WASHMOBILE PICHA7 MHEALTH AND CHLORINE E-VOUCHER PROGRAM IN THE DEMOCRATIC REPUBLIC OF THE CONGO

Jean-Claude Bisimwa[1], Presence Sanvura[1], Kelly Endres[2], Blessing M. Banywesize[1], Gisèle Nsimire[1], Feza Rugusha[1], Wivine Tumba[1], Pacifique Kitumaini[1], Claude Lunyelunye[1], Emmanuel Buhendwa[1], Pascal K. Bujiriri[1], Jessy Tumusifu[1], Brigitte Munyerenkana[1], Justin Bengehya[3], Jean-Claude Kulondwa[4], Ghislain Maheshe[1], Cirhuza Cikomola[1], Christine Marie George[5]

[1]Université Catholique de Bukavu, Bukavu, Democratic Republic of the Congo, [2]Baltimore, MD, United States, [3]Bureau de l'Information Sanitaire, Surveillance Epidémiologique et Recherche Scientifique, Division Provinciale de la Santé Sud Kivu, Ministère de la Santé, Bukavu, Democratic Republic of the Congo, [4]Division Provinciale de la Santé Sud Kivu, Ministère de la Santé, Bukavu, Democratic Republic of the Congo, [5]Johns Hopkins Bloomberg Sch of Pub Hlth, Baltimore, MD, United States

Background: Targeted WASH programs for those residing near to diarrhea patients can serve as a cost-effective approach during outbreaks to reduce the spread of diarrhea. We designed the WASHmobile program for those at high risk of diarrhea. In our randomized controlled trials in the DRC (PICHA7) and Bangladesh (CHoBI7), delivery of WASHmobile to diarrhea patient households through a healthcare facility visit, voice and SMS messages from a doctor significantly reduced diarrhea and improved child growth. Methods: Building on this work, we adapted WASHmobile to serve millions more beneficiaries through a mHealth and e-voucher program delivered in diarrhea outbreak areas. We evaluated this approach in health areas with ongoing diarrhea outbreaks in the DRC among 2022 participants. Voice and SMS messages were sent to those within 500 meters of diarrhea patients from a doctor stating that there was a diarrhea outbreak in their health area and emphasized the importance of treating and safely storing household drinking water and handwashing with soap for the next 7-day high-risk period. The SMS messages contained an e-voucher to redeem for free chlorine tablets at a pharmacy. Unannounced spot checks assessed WASH behaviors a week after program initiation. Results: Fifty-seven percent of WASHmobile households redeemed e-vouchers for chlorine tablets at pharmacies. Compared to control households, WASHmobile households that redeemed e-vouchers had higher stored drinking water with free chlorine concentrations >0.2 mg/L (Odds Ratio [OR]: 6.93, [95% Confidence Interval [CI]: 1.76, 27.24]) (64% [WASHmobile] vs. 20% [control]) and stored drinking water completely covered (OR: 4.55, 95% CI: 2.68, 7.70) (73% vs. 38%). Presence of a cleansing agent within 10 steps of latrine and cooking areas was also significantly higher in WASHmobile households (latrine: OR: 3.64, 95% CI: 1.47, 9.02 [70% vs. 39%] and cooking: OR: 2.50, 95% CI: 1.31, 4.77 [70% vs. 49%]). Conclusions The WASHmobile PICHA7 mHealth and e-voucher program significantly increased water treatment, safe water storage, and hygiene behaviors in diarrhea outbreak areas in the DRC.

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COMPARATIVE EVALUATION OF LABORATORY-BASED CONCENTRATION AND FILTRATION METHODS FOR WASTEWATER SURVEILLANCE OF ENTERIC PATHOGENS IN KARACHI, PAKISTAN

Farah Naz Qamar[1], Aneeta Hotwani[1], Furqan Kabir[1], Sonia Qureshi[2], Junaid Iqbal[3], Zarfishan Hussain[1], Wardah Mujahid[1]

[1]Aga Khan University, Karachi, Pakistan, [2]Aga Khan University Hospital, Karachi, Pakistan, [3]Aga Khan University, Cville, Pakistan

Wastewater-based surveillance is a cost-effective approach for monitoring community-level pathogens and provides early warning for outbreaks. However, accurate detection depends on efficient pathogen concentration and nucleic acid extraction methods in complex wastewater matrices. This study compared multiple workflows to identify optimal approaches for detecting enteric pathogens. Wastewater samples were collected from forty environmental surveillance sites across three towns between August and November 2021 and processed using different concentration and extraction methods, including Bag-Mediated Filtration System with total nucleic acid extraction (BMFS-TNA), Nanotrap-based methods (Nanotrap-RNA and Nanotrap-TNA), filtration techniques, and Dynabeads-based approaches. Detection performance was evaluated using customized TaqMan Array Cards targeting 77 viral, bacterial, and protozoal pathogens. BMFS-TNA demonstrated the highest overall sensitivity, particularly for viral detection. Nanotrap-based methods showed the most balanced and consistent performance across all pathogen groups, enabling reliable multi-pathogen detection. Filtration methods performed well for bacterial targets but showed variable viral recovery, while Dynabeads exhibited lower and less consistent performance. Overall, BMFS-TNA was most effective for viral detection, whereas Nanotrap methods provided a robust platform for broad pathogen surveillance. Further validation with larger sample sizes is needed to confirm these findings and support standardization of wastewater surveillance methods.

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A DIGITAL HEALTH TECHNOLOGY TO ADDRESS HEALTH DISPARITIES THROUGH IMPROVED SANITATION AND ENVIRONMENTAL HEALTH IN COMMUNITIES OF SALVADOR, BRAZIL

Fabiana Almerinda G. Palma[1], Mirella Ribeiro[1], Murilo Guerreiro Arouca[2], Anu Bourgeois[3], Claire Spears[4], Cassandra White[3], Joe Brown[5], Federico Costa[6], **Christine E. Stauber**[4]

[1]Colletive Health Institute, Federal University of Bahia, Salvador, Brazil, [2]Institute of Computing, Federal University of Bahia, Salvador, Brazil, [3]College of Arts and Sciences, Georgia State University, Atlanta, GA, United States, [4]School of Public Health, Georgia State University, Atlanta, GA, United States, [5]Department of Environmental Sciences and Engineering, Gillings School of Global Public Health, University of North Carolina-Chapel Hill, Chapel Hill, North Carolina, NC, United States, [6]Institute of Collective Health, Federal University of Bahia, Salvador, Brazil

Health disparities in sanitation increase the risk of infectious diseases and require community participation beyond infrastructure investments. Despite this, little research has examined the effects of participatory digital health technologies on health disparities, nor has it established best practices for equity-focused, user-centered implementation and evaluation of these technologies. We conducted a pilot feasibility study with a mixed-methods approach in two low-income communities (Nova Sussuarana and Marechal Rondon) in Salvador, Brazil, to assess the acceptability and feasibility of a citizen-participation program. The initial sample included 148 participants. The intervention used the Mais Lugar app for collaborative mapping of sanitation problems (photos, audio, and georeferencing submissions) and WhatsApp education messages delivered through a content-filtering recommendation system. To ensure digital equity, we provided mobile phones to 15% of participants and procedural instructions for using the app. Feasibility and acceptability were assessed via engagement rates, platform usage metrics, two surveys based on the Unified Theory of Acceptance and Use of Technology (UTAUT), and four focus groups (32 participants). The program achieved 82.4% retention, above the 75% target. A total of 139 sanitation problems were recorded, predominantly sewage leaks (41.7%). App engagement was high: 61.9% of the records had audio; 59% suggested solutions. The passive messaging system obtained 87% overall satisfaction. Surveys showed statistical growth across all evaluated dimensions, especially perceived ability to solve community problems (7.89 to 8.59 on a scale of 10) and the app's recommendation index (8.75 to 9.18). The qualitative stage confirmed high acceptance and potential to strengthen community empowerment. The program demonstrated high acceptability and feasibility in urban communities with a high risk of infectious diseases, serving as a promising tool to reduce health disparities and to guide the best practice, equity-centered, user-driven design of these technologies.

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FORMATIVE RESEARCH FOR ADAPTING THE CASE-AREA-TARGETED WASHMOBILE CHOBI7 INTERVENTION TO REDUCE CHOLERA IN URBAN BANGLADESH

Tahmina Parvin[1], Jahed Masud[1], Md Sazzadul Islam Bhuyian[1], Sawkat Sarwar[1], Jamie Perin[2], Ahmed Al-Kabir[3], Abu Syed Golam Faruque[4], Elizabeth Thomas[5], Peter John Winch[6], Christine Marie George[7], Fatema Zohura[1]

[1]Research, Training and Management International, Dhaka, Bangladesh, [2]Baltimore, MD, United States, [3]Research, Training and Management (RTM) International, Dhaka, Bangladesh, [4]icddr,b, Dhaka, Bangladesh, [5]Johns Hopkins Bloomberg School of Public Health, Arlington, VA, United States, [6]Johns Hopkins Bloomberg Sch of Public Health, Baltimore, MD, United States, [7]Johns Hopkins Bloomberg Sch of Pub Hlth, Baltimore, MD, United States

Cholera remains a major public health threat globally, causing an estimated 2.9 million cases and 95,000 deaths annually. Bangladesh is estimated to have over 160,000 cases each year with 84.5 million individuals residing in cholera-prone areas. Individuals residing within 20 meters of a cholera patient are at increased risk of cholera infections, particularly during the first month after patient hospitalization. However, there is limited evidence on targeted interventions for this high-risk population. To address this gap, we developed and piloted the WASHmobile CHoBI7 case-area-targeted intervention (CATI), integrating water, sanitation, and hygiene (WASH) and mobile health, to reduce cholera transmission in urban informal settlements in Dhaka. Culture-confirmed cholera patients were recruited from icddr,b Dhaka Hospital and their households and neighboring compounds were enrolled across five iterative pilot phases of 1475 participants. A mixed-methods evaluation was conducted including 61 semi-structured interviews and five focus group discussions. The WASHmobile program included a compound-level ring session and group sessions, WASH kit distribution, and weekly voice and text messages. Outcomes were assessed through structured observations and unannounced spot checks over three months, compared with a standard arm receiving only the standard message on using oral rehydration solution. In the final phase of piloting, handwashing with soap was significantly higher in the WASHmobile arm at Day 7 [79% vs. 9%, $p<0.0001$] and Month 1 [89% vs. 12%, $p<0.0001$], as well as for stored drinking water with free chlorine >0.2 mg/L at Day 7 [74% vs. 13%, $p<0.0001$] and Month 1 [56% vs. 12%, $p<0.0001$]. Facilitators of WASHmobile delivery included a compound-level soapy water donation systems and frequent mobile messaging, while barriers included misconceptions about chlorine tablets and limited mobile message sharing. Overall, the WASHmobile CHoBI7-CATI program had high acceptability, feasibility, and adoption, presenting a promising approach to increase WASH behaviors among high-risk populations for cholera.

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IMPROVED, HIGH-RESOLUTION ESTIMATES OF WATER, SANITATION, AND HYGIENE ACCESS IN LOW- AND MIDDLE-INCOME COUNTRIES (LMICS).: A SPATIALLY REFERENCED HIERARCHICAL, ORDINAL MULTINOMIAL MODELING ANALYSIS

Bin Fang[1], Venkataraman Lakshmi[1], Margaret N. Kosek[2], **Josh Michael Colston**[3]

[1]UVA School of Engineering, Charlottesville, VA, United States, [2]University of Virginia, Charlottesville, VA, United States, [3]UVA School of Medicine, Charlottesville, VA, United States

Access to safe drinking water, improved sanitation, and basic hygiene (WASH) are determinants of health outcomes like infectious diseases, malnutrition, gender-based violence, and mortality, particularly in low- and middle-income countries (LMICs). Even within particular LMICs, WASH access is highly heterogeneous, with sub-national and sub-regional inequalities that are masked by aggregate statistics. In this study from the Planetary Child Health & Enterics Observatory (Plan-EO), we leverage recent improvements in geostatistical software and methods to model coverage of each category of the three WASH component variables, and provide the stakeholder community with globally consistent, high-resolution datasets of the resulting predictions. We compiled and georeferenced data on household-level WASH conditions from 359 nationally representative surveys carried out since 2005 in 107 LMICs, representing over 6 million households, and extracted data from 24 environmental and sociodemographic variables at 291,000 cluster locations. Spatially explicit ordinal multinomial regression models were fitted using R Template Model Builder (RTMB), incorporating cluster-level random effects and spatial random fields represented by the stochastic partial differential equation (SPDE) formulation. Large regions of low coverage of the most improved WASH categories were predicted across Sub-Saharan Africa, South and Southeast Asia (except for sanitation in Thailand), Amazonian South America, Western China and Mongolia. While human development index and nighttime lights were important predictors across all three WASH components, other predictors ranked higher for just one component such as distance to wastewater treatment plant for sanitation, temperature range for water, and irrigation areas for hygiene, indicating specificity to the modeled associations. AUC statistics indicated excellent discrimination for

most categories in the models particularly piped water sources (0.91) and sewer or septic tank sanitation (0.91). The resulting datasets will be made available via a public data repository once published.

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CONTINUUM FROM INTERMITTENT TO CONTINUOUS WATER SUPPLY: VARIATION IN SUPPLY FREQUENCY AND DURATION AND ASSOCIATIONS WITH WATERBORNE INFECTIONS IN HUBLI-DHARWAD, INDIA

Ayse Ercumen[1], Zachary Burt[2], Sharada Prasad[3], Narayan Billava[4], Nayanatara Nayak[4], Emily Kumpel[5]

[1]North Carolina State University, Raleigh, NC, United States, [2]California Department of Water Resources, Sacramento, CA, United States, [3]Infosys, Bengaluru, India, [4]Center for Multidisciplinary Development Research, Dharwad, India, [5]University of Massachusetts, Amherts, Amherst, MA, United States

Intermittent water supplies (IWS) serve >1 billion people globally and can compromise water quality and transmit waterborne infections. Supply frequency and duration vary between and within IWS systems. The UN Sustainable Development Goals target having water "available when needed" for ≥12 hours/day or ≥4 days/week but there are scarce data on how supply frequency and duration within IWS affect health outcomes. We conducted a matched study in Hubli-Dharwad, India, a city served partially by continuous water supply (CWS) and partially by IWS, to assess associations between water supply characteristics and waterborne infections. We enrolled 2220 CWS households and 2218 IWS households matched on socioeconomic and sanitation conditions. We compared diarrhea prevalence in children <5 years and typhoid fever incidence between IWS households with different water supply frequency/duration and CWS households using generalized linear models with robust standard errors and adjusting for socio-demographics and sanitation access. Among IWS households, the median supply frequency was every 8 days (interquartile range [IQR]=7-8), and the median supply duration was 4 hours (IQR=3-5). IWS households had 36% higher prevalence of child diarrhea (PR=1.36, 1.00-1.84) and 78% more typhoid fever cases (CIR=1.78, 1.11-2.84) than CWS households. IWS households in the bottom quintiles of supply frequency and duration (receiving water once every 9-15 days or for <3 hours per supply cycle) had 45-72% higher prevalence of child diarrhea and 2 times as many typhoid fever cases (p-values<0.05). In contrast, in IWS households in the upper quintiles of supply frequency and duration (receiving water at shorter intervals and/or for more hours per supply cycle), waterborne risks were broadly indistinguishable from CWS households. Our findings support global efforts to implement CWS but also highlight that increasing supply frequency and duration in IWS systems in the interim can deliver health benefits.

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ANTIBODY RESPONSES TO *IXODES SCAPULARIS* SALIVARY PROTEINS AS BIOMARKERS OF TICK EXPOSURE IN A MULTI-SITE U.S. COHORT

Madiha Shafquat[1], Luana Rogerio[2], John Glenn Morris[3], Anna P. Durbin[1], Albert Icksang Ko[4], Adam Waickman[5], L Hannah Gould[6], Adriana Gallagher[3], Patrick Kelly[6], Lucas Tirloni[7], Juanita Edwards[6], Cristina Hladish[3], Ross M. Boyce[8], Shelley Kon[9], Suhyun S. An[4], Paloma Carcamo[4], Tiago D. Serafim[2], Ronja Frigard[2], William Gregory[5], Rachel Hampton[1], Eva Iniguez[2], Michelle Klick[5], Peter J. Krause[4], Alexandra Loew-Baselli[6], Maureen Long[3], Aline DS Moreira[2], Lina Moscarella[1], M. Catherine Muenker[4], Nivison Nery Jr.[4], Kristopher Paolino[5], Ruiyu Pu[3], Vagner Dias Raimundo[2], Beulah P. Sabundayo[1], Cyrus Saleem[3], Sarah Sampson[1], Luke Trimmer-Smith[3], Jesus G. Valenzuela[2], Derek AT Cummings[1]

[1]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [2]National Institutes of Health, Rockville, MD, United States, [3]University of Florida, Gainesville, FL, United States, [4]Yale School of Public Health, New Haven, CT, United States, [5]State University of New York Upstate Medical University, Syracuse, NY, United States, [6]Pfizer, New York, NY, United States, [7]National Institutes of Health, Hamilton, MT, United States, [8]University of North Carolina Chapel Hill, Chapel Hill, NC, United States, [9]University of Colorado School of Medicine, Aurora, CO, United States

Understanding human exposure to tick bites is critical for assessing risk of infection with *Borrelia burgdorferi* sensu lato (Bbsl, the causative agent of Lyme disease), and other tick-borne infections. The host mounts antibody responses to salivary proteins released by ticks during feeding, which may serve as biomarkers of tick exposure and predictors of Bbsl infection risk. We conducted a prospective cohort study at four US sites with differing *Ixodes scapularis* exposure risk (Connecticut, New York, Maryland, Florida). Serum samples from participants at each site were collected in 2024 before (May–June) and after peak nymphal season (October–December). Antibodies to *I. scapularis* and Bbsl were measured by ELISA using *I. scapularis* salivary proteins and VlsE–OspC as antigens, respectively. Questionnaires captured demographics, tick encounters, outdoor activity, protective behaviors, and environmental risk factors. Associations between salivary antibody responses, covariates, and Bbsl serostatus were evaluated. Among 805 participants, higher mean salivary antibody responses were significantly associated with study site, age, sex, reported tick encounters, yard characteristics, and Bbsl serostatus. Increases between visits differed by site, with Connecticut participants showing substantially higher odds than Florida (Odds Ratio (OR) 3.2, 95% CI 2.0–5.3), Maryland (OR 4.8, 2.7–8.3), or New York (OR 4.2, 2.6–6.7). Salivary antibody level and site were the strongest predictors of Bbsl seropositivity; individuals at the 95th percentile of response had 4.37-fold higher odds (95% CI 3.40–5.56) than those at the 5th percentile. Antibody responses to *I. scapularis* salivary proteins over a single season and their change over time correlate with known tick-exposure risks and Bbsl serostatus, supporting their use as biomarkers of tick exposure.

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BIOMARKERS OF *BABESIA MICROTI* INFECTION AND SEVERITY OF CLINICAL BABESIOSIS

Miranda SM Oakley, Hong Zheng, Sanjai Kumar

FDA/CBER/OBRR, Silver Spring, MD, United States

Human babesiosis, caused by the intraerythrocytic parasite *Babesia microti*, presents a significant public health burden in the United States and globally. The immunology and pathogenesis of human babesiosis is poorly understood. Based on a series of studies in a B cell deficient patient who had experienced three clinical episodes of babesiosis during a six-year period, and in 14 hospitalized patients diagnosed with a spectrum of clinical babesiosis, we have identified novel biomarkers that shed light on reinfection immunity to *B. microti* and have identified immunological correlates of parasite burden and disease severity. Our studies reveal several striking findings. The B cell deficient patient had exceptionally high *Babesia microti* specific antibodies despite very few circulating B cells which predominantly co-expressed CD27 (memory marker) and CD95 (death receptor). This patient also had long-lasting NK cells and expansion of stem memory T cells. In addition, the patient's levels of the IP-10 cytokine directly correlated with parasite burden. In the case series, babesiosis patients exhibited enhanced expression of eight cytokines (eotaxin, IL-6, IL-8, IL-10, IP-10/CXCL10, MCP-1, MIP-1α, and TNF-α) compared to uninfected controls. In addition, circulating IP-10/CXCL10 correlated with both parasite burden of *B. microti* and disease severity of babesiosis. Cumulatively, our results showed that IP-10 levels may serve as a reliable marker of *B. microti* parasite burden and the severity of clinical babesiosis.

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INTEGRATING HUMAN MOBILITY AND ACTIVITY PATTERNS WITH RISK PERCEPTION INTO A SPATIAL ENVIRONMENTAL RISK ASSESSMENT FRAMEWORK FOR TICKS IN STATEN ISLAND, NEW YORK

Maria del Pilar Fernandez[1], Maria Diuk-Wasser[2], Danning Ma[2], Wen Fu[2], Maximilian Mason[3]

[1]Washington State University, Pullman, WA, United States, [2]Columbia University, New York, NY, United States, [3]Northwestern University, Evanston, IL, United States

Environmental risk assessments for ticks often focus on ecological indicators such as habitat suitability or tick and tick-borne pathogen occurrence, but these measures do not capture how people move through landscapes, what activities they engage in, or how they perceive disease risk, which will ultimately affect human-tick encounters. We evaluated these components using three human-centered data streams collected on Staten Island, New York, during 2019-2021 as part of a filed study to evaluate tick-borne disease dynamics in urban areas. We used data derived from the Tick App, a smartphone app designed to capture human behaviors related to tick-borne diseases. We first developed a standardized pipeline for preprocessing, validating, and analyzing GPS trajectory data derived from the app, including spatial-temporal filtering combined with high-resolution land cover data, to quantify time spent in environmentally relevant spaces. We then summarized daily survey data submitted via the app, to evaluate reported activities, use of personal protective measures, and self-reported tick encounters over time to evaluate the frequency of risky activities and their association with preventative measures. Lastly, to assess individual risk perception, we analyzed data from a field-based Knowledge, Attitudes and Practices survey using a Bayesian Item Response Theory cumulative logit model to estimate a latent Lyme disease risk perception score. Preliminary risk perception analyses showed that personal or proximal Lyme disease experience was associated with higher latent risk perception scores, which explained 13% of the variation in risk perception, and inclusion of behavioral variables modestly improved explanatory power to 17%. The individual-based information derived from the three data streams will be used to create a composite socio-behavioral layer at the neighborhood scale using a factor analysis pipeline. This layer could ultimately be integrated with a tick hazard to provide more comprehensive socio-ecological risk assessment for tick-borne diseases.

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CLINICALLY VALIDATED ASSAYS FOR LYME SEROLOGY IN EPIDEMIOLOGICAL APPLICATIONS

Cara Martone[1], Sam R. Telford[1], Karen Weeks[2]

[1]Tufts University, Department of Infectious Disease & Global Health, North Grafton, MA, United States, [2]Eco Laboratory, Acton, MA, United States

For serological confirmation of a diagnosis of Lyme disease, the CDC currently recommends either the standard two-tier testing (STTT) with an enzyme immunoassay (EIA) screening followed by a confirmatory immunoblot (WB) for suspected positives, or a modified two-tier testing (MTTT) using two EIAs. Such assays must be conducted in the context of clinical manifestations and pretest probabilities. For epidemiological studies, focus on prevalent cases may not have a clinical context. We determined the concordance of STTT and MTTT assays with self-reports of disease. Subjects were enrolled from 4 New England island communities endemic for LD. Serum samples and a screening questionnaire were obtained. Serology was conducted using STTT (IgG capture EIA followed by confirmation with an FDA-cleared commercial line immunoblot with recombinant antigens) and an FDA-cleared MTTT kit for IgG. Exposure prevalence was 18% (95% CI 15-22%) by STTT, 46% (95% CI 39-54%) by the commercial MTTT kit. 40% of the subjects self-reported a history of LD (95% CI 35-45%). Given the unusual prevalence estimates from the MTTT and self-reports, recombinant line blots were conducted on all samples regardless of first-tier EIA results. Those that were considered MTTT positive but STTT negative demonstrated reactivity to 2-5 *Borrelia burgdorferi*-specific antigens (93,58, 39, 23,18kDa) as well as reactivity to the cross-reactive flagellin 41kDa. We analyzed questionnaires from those with a negative STTT EIA result and a MTTT positive result, and found that many (42%, 95% CI 20-67%) reported past LD >10 years prior. It is likely that in populations with decades of intense deer tick exposure (high pretest probability) there are individuals with a long-prior history of LD and a waning antibody response that may not be detected by typical STTT. We conclude that there is a general epidemiological question posed by the significance of evidence of historical exposure, and underscore the need to contextualize assay choice and interpretation with specific study objectives.

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PRE-EXISTING HOST IMMUNITY RESTRICTS TICK CO-FEEDING TRANSMISSION OF FLAVIVIRUSES

Sarah C. Macon-Foley[1], Clemence Obellianne[2], Meghan Hermance[1]

[1]University of South Alabama, Mobile, AL, United States, [2]Mobile, AL, United States

Tick co-feeding transmission occurs when infected and uninfected ticks feed simultaneously on the same vertebrate host, enabling virus transfer between ticks without requiring host viremia. This mechanism is thought to be critical for maintaining many tick-borne viruses (TBVs) in nature. Although antibodies against several TBVs have been detected in wildlife species, indicating previous exposure, how vertebrate host immune status impacts tick co-feeding transmission of TBVs remains poorly understood. To investigate this, we used an *in vivo* tick transmission model consisting of Langat virus (LGTV)-infected *Ixodes scapularis* nymphs ("donors") and pathogen-free *I. scapularis* larvae ("recipients") co-feeding on either virus- and tick-naïve mice or LGTV-immune mice. After feeding to repletion, donors and recipients were individually screened for LGTV RNA via q-RT-PCR. Co-feeding transmission efficiency, defined as the proportion of recipient ticks acquiring detectable viral RNA, was significantly reduced on virus-immune mice compared to the naïve mice (Chi-Square, p-value <0.0001). Transmission efficiency also differed by immunization strategy: mice immunized by infected tick bite exhibited significantly higher transmission efficiency than mice immunized by intraperitoneal LGTV injection (Fisher's exact test, p-value = 0.0279). Despite these differences in transmission efficiency, viral RNA levels in infected recipient ticks did not differ significantly among treatment groups. To investigate cutaneous mechanisms underlying the differences in co-feeding transmission efficiency, single-cell RNA sequencing and flow cytometry of the tick co-feeding site are underway. Skin from virus-naïve and virus-immune mice will be assessed at 1 and 3 days of co-feeding between LGTV-infected or mock-infected donors and pathogen-free recipients. Together, these studies will define how vertebrate host viral immunity reshapes the cutaneous microenvironment during tick co-feeding and identify host immune pathways influencing viral transmission among co-feeding ticks.

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THE ROLE OF A SPECIFIC GROUP OF MEMORY T CELLS IN VACCINATION-INDUCED PROTECTION AGAINST RICKETTSIAE

Loka Reddy Velatooru Reddy Velatooru[1], Garrett Cutchin[1], Nicole Burkhardt[2], Bing Zhu[1], Yingzi Cong[3], Haiping Hao[1], David H. Walker[4], Ulrike Gertrud Munderloh[5], **Rong Fang**[1]

[1]UTMB, Galveston, TX, United States, [2]University of Minnesota, St. Paul, MN, United States, [3]Northwestern University, Chicago, IL, United States, [4]University of Texas Medical Branch, Galveston, TX, United States, [5]University of Minnesota, Saint Paul, MN, United States

Spotted fever rickettsioses, which are mainly transmitted by ticks, can be fatal in otherwise healthy individuals. No licensed vaccine currently exists. We recently reported that *Rickettsia parkeri* mutant 3A2 confers complete protection against *R. parkeri* rickettsiosis as a live-attenuated vaccine candidate. In the present study, we seek to reveal the contribution of $CD4^+$ and $CD8^+$ T cells to 3A2-induced long-lasting protection, as well as the molecular basis involved. Compared to mock immunization, after one

year immunization, 3A2 elicited a significant increase in the frequency of $CD44^+CD62L^-$ effector memory $CD8^+$ T cells producing *R. parkeri*-specific IFN-gamma in blood, which was associated with durable protection against a rickettsial lethal challenge. This was not observed in $CD4^+$ T cells. To further uncover the role of specific T cell subsets in immune correlates of protection, we depleted over 90% of CD4 and/or $CD8^+$ T cells by specific antibodies. Interestingly, upon a lethal rickettsial challenge, 3A2-immunized mice with $CD8^+$ T cell depletion showed a significant decrease in host survival of over 50% and an increase in rickettsial load in liver, along with a reduction in circulating IFN-gamma levels compared to non-depleted controls. However, depletion of $CD4^+$ T cells did not result in any significant changes. In addition, adoptively transfer of purified $CD8^+$ T cells of 3A2-immunized WT mice conferred complete protection to C3H-SCID mice against a lethal rickettsial challenge. $CD4^+$ T cells, on the other hand, provided only 50% protection. The transcriptomic analysis of purified $CD8^+$ T cells from 3A2-immunized mice revealed a well-established memory $CD8^+$ T cell program, as indicated by increased expression of survival markers such as IL7-R and BCL2, as well as memory-associated transcription factors like TCF7, EOMES, and T-bet. Our research collectively showed that $CD8^+$ memory T cells represent an immune correlate of protection against rickettsiae.

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EARLY IMMUNE SIGNALING RESPONSES IN *HAEMAPHYSALIS LONGICORNIS*: EFFECTS OF BLOOD FEEDING AND SFTSV INFECTION

Eliane Esteves, Margaret G. Schuler, Meghan Hermance
University of South Alabama, Mobile, AL, United States

Severe fever with thrombocytopenia syndrome virus (SFTSV) is an emerging tick-borne virus of increasing public health concern. *Haemaphysalis longicornis*, commonly known as the Asian longhorned tick, is a primary vector; however, the immune responses underlying early tick-virus interactions and the influence of blood feeding remain poorly understood. In this study, we investigated the modulation of key immune signaling pathways in *H. longicornis* nymphs in response to blood feeding and SFTSV infection, with a particular focus on early time points. To distinguish the effects of feeding and SFTSV infection, we evaluated the expression of core components of major immune signaling pathways, including Spätzle and Dorsal (Toll pathway), STAT (JAK/STAT pathway), and Relish (IMD pathway), under two conditions: **i**) fed versus unfed nymphs at early time points (1 to 3 days post-attachment [d.p.a.]) and **ii**) SFTSV-infected versus mock-infected nymphs under fed conditions at early stages of infection (1 to 3 days post-infection [d.p.i.]). Our results demonstrate that blood feeding modulates immune signaling in uninfected ticks, with upregulation of Dorsal, STAT, and Relish at 3 d.p.a., while Spätzle expression was not detected. In contrast, SFTSV infection in fed ticks induces a rapid and coordinated upregulation of all evaluated genes, particularly at 2 d.p.i. This early activation contrasts with more variable expression at later stages, indicating dynamic immune regulation during SFTSV infection. These findings highlight the importance of physiological state and infection status in shaping tick immune responses and identify early time points as a critical window for tick-pathogen interactions. Ongoing studies will investigate whether antimicrobial peptides (AMP's) gene expression is modulated by blood feeding and infection, and future work will explore the functional roles of key immune signaling genes using RNA interference (RNAi). Together, these results provide new insights into early immune responses and molecular interactions between *H. longicornis* and SFTSV, contributing to a better understanding of tick innate immunity.

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MOXIDECTIN FOR SCABIES: DOSE CONFIRMATION IN A PHASE 2, PLACEBO CONTROLLED, RANDOMIZED EFFICACY AND SAFETY STUDY OF SINGLE ORAL DOSES IN ADULTS WITH SCABIES

Laura Vargas Rivas[1], Daisy Blanco[2], Jorge Lopez[3], Patricia Zuniga[4], Armando Pineda Velez[5], Bruce Torkan[6], Gilberto Perez[7], Jorge Castillo Molina[8], Richard Fernandez[9], Moraye Bear[10], Vidya Uprety[11], Mark Sullivan[11], **Victoria Ryg-Cornejo**[11]
[1]Vargas' Clinic, San Salvador, El Salvador, [2]Instituto Dermatologico Dominicano y Cirugia de Piel. "Dr. Huberto Bogaert Diaz", Santo Domingo, Dominican Republic, [3]Hospital y Clinica Bendana, San Pedro Sula, Honduras, [4]DerClinic, San Pedro Sula, Honduras, [5]Medical Research of Westchester, Miami, FL, United States, [6]LA Universal Research Center, Los Angeles, CA, United States, [7]Evolution Clinical Trials, Miami, FL, United States, [8]Affinity Clinical Research, Tampa, FL, United States, [9]Advance Care and Clinical Trials, Miami, FL, United States, [10]Forsythe and Bear LLC, Los Angeles County, CA, United States, [11]Medicines Development for Global Health, Southbank, Australia

Scabies is one of the world's most common contagious skin conditions. Far from a benign condition, scabies can drive a cascade of serious complications that include bacterial infections, kidney disease and rheumatic heart disease, contributing to substantial but under-recognized morbidity and mortality and pressure on healthcare systems. Adherence to treatment is essential for effectiveness, but most existing treatments are cumbersome to use because of topical application challenges or repeat dose regimens, so there is an unmet need for a new oral treatment with simpler dosing. We conducted a Phase 2, multinational, multi-center, randomized, double-blind, placebo controlled study to evaluate the efficacy and safety of single oral doses of 8, 16, or 32 mg moxidectin compared to placebo in adults with scabies. Two hundred participants were randomized from sites in the Dominican Republic, El Salvador, Honduras and the United States in an equal allocation ratio. The primary efficacy endpoint of Complete Cure is defined as a combination of clinical cure (all clinical features of the infestation have resolved including pruritus) and microscopic or dermatoscopic cure (absence of mites and/or mite products and burrows) 28 days after treatment. The Complete Cure rate for 8 (n=50), 16 (n=49) and 32 mg moxidectin (n=51) was 56.0% (95% CI 41.3, 70.0), 63.3% (48.3, 76.6) and 62.7% (48.1, 75.9), respectively, compared to 2% (0.1, 10.9) (n=49) in the placebo group (p-value for the risk difference <0.0001). Therefore, the 3 dose levels of moxidectin had a similar treatment effect. The primary analysis was supported by exploratory efficacy analyses including an Investigator Assessment of Cure. Safety was assessed up to 16 weeks post-treatment. Moxidectin was well tolerated, with no clear dose-related trends in safety findings and a similar safety profile to placebo. Thirty-three (16.6%) subjects overall reported adverse events; the most common AEs were headache (n=7 [3.5%]), diarrhea (n=7 [3.5%]), and influenza (n=6 [3%]). These data support a single oral moxidectin 8 mg dose as a candidate treatment for scabies in adults.

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NO CASES, NO RISK? DETECTION OF HEARTLAND VIRUS IN *AMBLYOMMA AMERICANUM* NYMPHS IN SOUTH CAROLINA

Emily E. Owens Pickle[1], Madeleine Meyer Torelli[1], Diana Zychowski[2], Sean Patrick Sweeney[1], Kyndall Dye-Braumuller[1], Stella Self[1], Kia Zellars[1], Ross M. Boyce[3], Scott P. Commins[4], Melissa Nolan[1]
[1]University of South Carolina, Columbia, SC, United States, [2]University of North Carolina, Chapel Hill, NC, United States, [3]University of North Carolina Chapel Hill, Chapel Hill, NC, United States, [4]University of North Carolina at Chapel Hill, Chapel Hill, NC, United States

Heartland virus (HRTV) and Bourbon virus (BRBV) are emerging tick-borne pathogens associated with severe human illness in the United States of America. South Carolina lies within the range of the primary vector for both viruses, *Amblyomma americanum* L. 1758, and vertebrate serologic

evidence suggests regional circulation. However, no human cases have been reported in the state. We conducted statewide surveillance of host-seeking *A. americanum* nymphs collected from April 2021 to August 2024 using standard methods. Ticks were morphologically identified, bisected, and then pooled after RNA extraction. Viral RNA was detected using optimized RT-PCR assays targeting HRTV and BRBV. A total of 1,665 nymphs were tested in pools of ≤5 nymphs; six pools (6/368) were positive for HRTV and none were positive for BRBV. All HRTV-positive pools originated from the southern coastal ecoregion. Positive pools were collected in April, July and August. Positive pools comprised nymphs collected in 2021 and 2022. All positive pools also showed detection for spotted fever group *Rickettsia* (panrickettsia). In Conclusion, detection of HRTV in nymphal ticks from coastal South Carolina demonstrates local viral circulation in a region without reported human cases. This finding highlights a possible disconnect between risk and reported cases, with potential for spillover or covert transmission. Enhanced surveillance and increased clinical awareness are needed to identify and mitigate this and other emerging tick-borne illnesses in tick-endemic regions.

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EPIDEMIOLOGICAL AND CLINICAL CHARACTERISTICS OF HANTAVIRUS CARDIOPULMONARY SYNDROME IN BRAZIL FROM 1993 TO 2025

Xinyi Hua[1], Ingra M. Claro[1], Amay Y. Xu[2], Julia Forato[3], José Luiz Proença-Modena[3], William M. de Souza[1]

[1]University of Kentucky, Lexington, KY, United States, [2]Paul Laurence Dunbar High School, Lexington, KY, United States, [3]University of Campinas, Campinas, Brazil

Hantaviruses are emerging rodent-borne pathogens that cause severe and often fatal disease in humans nearly worldwide. These include hemorrhagic fever with renal syndrome (HFRS) in Europe and Asia, and hantavirus cardiopulmonary syndrome (HCPS) in the Americas. In this study, we integrated epidemiological, ecological, clinical, and genomic data to investigate the transmission dynamics and genetic diversity of HCPS in Brazil. Between 1993 and 2025, a total of 2,472 HCPS cases were reported with a national-level cumulative case fatality rate (CFR) of 38.7% (957 of 2,472). The median age of HCPS patients was 35 years (interquartile range=25-45), and 77.1% were male. No significant differences in HCPS incidence and mortality rates by age group were observed. Rural municipalities had a 6.6-fold higher incidence than urban municipalities. Notably, we found dyspnea, shock, and cardiac symptoms were significantly more frequent in HCPS cases than in hantavirus-negative individuals and among fatal HCPS patients compared to survivors (χ^2 tests, all $p<0.001$). Agricultural and forest-related work was strongly associated with testing positive for hantavirus (adjusted Odds Ratio (aOR) 1.7, 95%CI 1.5-2.0), while dyspnea (aOR 3.2, 95%CI 2.3-4.5) and shock (aOR 3.3, 95%CI 2.5-4.4) were strong predictors of death among infected patients. Next, we recovered a nearly complete trisegmented genome comprising S (1,839 nt), M (3,818 nt), and L (6,446 nt) segments from a fatal HCPS case in São Paulo State, where the CFR is 54%. Phylogenetic analysis showed that this virus clusters with the Andes virus, sharing 92.5% and 93.2% amino acid identity across all segments. Based on ecological, epidemiological, and taxonomic criteria, we propose this as a novel Orthohantavirus species: Araraquara virus, which appears to be associated with a higher CFR among hantaviruses. Collectively, these findings support risk-based surveillance, guide community education and rodent control efforts, and inform targeted resource allocation to ensure timely clinical care.

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THE HIDDEN BURDEN OF CHIKUNGUNYA: 10-YEAR INCIDENCE OF MUSCULOSKELETAL DISORDERS IN A PEDIATRIC POPULATION

Jose Victor Zambrana[1], Sonia Arguello[2], Damaris Collado[2], Luis Nery Sanchez[2], Sergio Ojeda[2], Miguel Plazaola[2], Guillermina Kuan[2], Aubree Gordon[3], Angel Balmaseda[2], Eva Harris[4]

[1]Sustainable Sciences Institute, Oakland, CA, United States, [2]Sustainable Sciences Institute, Managua, Nicaragua, [3]Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor, MI, United States, [4]Division of Infectious Diseases and Vaccinology, School of Public Health, University of California, Berkeley, Berkeley, CA, United States

Chikungunya virus (CHIKV) is a reemerging global public health threat. While it is well known to cause short- and medium-term chronic musculoskeletal (MSK) symptoms in adults, and to a lesser degree in children, the extent to which these symptoms persist long-term in pediatric populations remains largely unknown. We analyzed longitudinal data from the long-standing Pediatric Dengue Cohort Study in Managua, Nicaragua. We followed participants with confirmed CHIKV infections (350 symptomatic and 480 inapparent infections) and 3,491 uninfected controls from 2016 to 2025 (ages 2-17). We evaluated the 10-year incidence of MSK disorders documented as the primary reason for clinical visits. Time-to-event analysis was conducted using an Accelerated Failure Time (AFT) model, adjusting for age, sex, and healthcare utilization patterns. We observed a distinct gradient effect in the long-term incidence of MSK disorders. Compared to uninfected controls, time to MSK diagnosis was significantly accelerated by 29% following symptomatic CHIKV infection (Time Ratio = 0.71, p = 0.024) and by 15% following inapparent infection (Time Ratio = 0.85, p = 0.394). Pain in joint and low back pain were the most common diagnoses across groups. While the majority of the separation between groups occurred during the first few years post-infection, a gradual and continuous separation was observed throughout the entire 10-year period, showing a sustained, higher susceptibility among CHIKV-infected symptomatic individuals. Our findings reveal a hidden burden of MSK disorders in pediatric populations lasting up to a decade following a CHIKV epidemic. This persistent morbidity supports the development of prophylactic CHIKV vaccines to prevent extended childhood disability.

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DEVELOPMENT OF A MUNICIPALITY-LEVEL EARLY WARNING ALERT AND RESPONSE SYSTEM FOR DENGUE CONTROL IN PUERTO RICO USING A BAYESIAN SPATIOTEMPORAL MODEL

Carol Liu[1], Ariana Lee Detmar[2], Zachary Madewell[3], Forrest Jones[1], Yashira Maldonado[1], Jomil Torres Aponte[4], Melissa Marzan-Rodriguez[1], Gabriela Paz-Bailey[5], Laura E. Adams[1], Maile Thayer[6]

[1]Centers for Disease Control, San Juan, PR, United States, [2]Alakaina, San Juan, PR, United States, [3]San Juan, PR, United States, [4]Puerto Rico Department of Public Health, San Juan, PR, United States, [5]CDC Dengue Branch, San Juan, PR, United States, [6]Centers for Disease Control and Prevention, San Juan, PR, United States

Dengue transmission is often localized in time and space. In Puerto Rico, outbreak response efforts are targeted and resource intensive. Short-term predictions of localized outbreak probability can support operational decision-making and situational awareness for dengue response. We developed and evaluated a municipality-level early warning alert and response system using a Bayesian hierarchical spatiotemporal model fit to weekly dengue case data from 1986 to 2024 for all 78 municipalities. The base model included seasonal, annual and spatial random effects. We assessed the incremental explanatory value of adding climatic covariates, specified as distributed non-linear lags, and rolling estimates of population-level dengue susceptibility. We found that the Standardized Precipitation Evapotranspiration Index (SPEI; drought index) and minimum temperature contributed the most to dengue prediction. This model was used to generate municipality-level forecasts at 1-12-week horizons.

Outputs were expected case counts and the probability of exceeding a municipality threshold defined as the 90th percentile of historical values. Out-of-sample validation assessed the model's ability to predict weeks of threshold exceedance, compared to observed outcomes, using AUC (score of 0.5 indicates random guessing, while 1.0 represents perfect identification of threshold exceedance), sensitivity and false alarm rate. At a 4-week forecast horizon, model predictions achieved strong discriminatory performance, with an average municipality-level AUC of 0.90 (range: 0.75-0.96), sensitivity of 88% (range: 62% -100%) and false alarm rate of 76% (range: 51-97%). Performance remained high at 8- and 12-week horizons, with average AUCs of 0.86 and 0.83. The model performed similarly well in municipalities with high-, medium- and low- incidence. Ongoing work focuses on visualizing changing outbreak risk at the municipality-level and using risk estimates to guide priority response locations when case clusters are detected. The aim is to translate probabilistic risk signals into clear, operational decisions under resource constraints.

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MATERNAL ANTIBODY KINETICS TO DENGUE AND ZIKA VIRUS DIFFER BY MOTHER'S INFECTION HISTORY

Matt Hitchings[1], Luke Trimmer-Smith[2], Albert Icksang Ko[3], Derek Cummings[4]

[1]University of Florida, Gainesville, FL, United States, [2]Gainesville, FL, United States, [3]Yale School of Public Health, New Haven, CT, United States, [4]Johns Hopkins University, Baltimore, MD, United States

Maternal antibodies to dengue virus (DENV) contribute to the risk of severe disease in infants through antibody-dependent enhancement. Evidence from epidemiological studies and birth cohorts in Southeast Asia and Brazil has suggested that infant risk may differ depending on whether their mother has experienced one or multiple previous dengue infections (monotypic vs. multitypic). However, no direct comparison of serological profiles has been made between infants born to dengue monotypic and multitypic mothers in the same setting, nor of how the kinetics are affected by history of infection with Zika virus (ZIKV). We conducted a secondary analysis of serum samples from mother-infant pairs enrolled in the Zika in Pregnancy (ZIP) study in Salvador, Brazil in 2016-2018. Using focus reduction neutralization tests (FRNT), we measured neutralizing antibody titer to four serotypes of DENV (1 to 4) and to ZIKV in 47 mother-infant pairs, with an average of four samples per infant from birth to 12 months. We describe the serological profile of mothers and use a Bayesian hierarchical model to estimate virus- and serotype-specific transfer and decay of maternal antibodies. A total of 36 mothers showed evidence of multiple previous DENV infections, five were classified as DENV-3 monotypic, and six as DENV-4 monotypic. In addition, 39 mothers had neutralizing titer to ZIKV. The correlation between maternal and birth titers was moderate (Pearson's correlations 0.28 to 0.82 by virus). Infants born to multitypic mothers had higher average FRNT titer at birth (median average 224 vs. 48.5) and at three months (36.4 vs. <20), but by six months the FRNT titers were similar between the two groups. The estimated half-life of maternal antibodies was 25-30 days, similar to a previous estimate of 28 days from a Thai cohort. Infant DENV serological profile differs by maternal infection history, suggesting that the time window of severe disease may also differ. With the increasing spread of dengue to immunologically naïve populations and the availability of vaccination, serostatus of mothers should be considered in the management and surveillance of infant dengue.

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DENGUE VIRUS SEROTYPE 3 LINEAGE REPLACEMENT IN NICARAGUA IS ASSOCIATED WITH REDUCED DISEASE SEVERITY

Miguel Plazaola Cuadra[1], Jose Victor Zambrana[2], Carlos Montenegro[1], Cristhiam Cerpas[1], Gerald Vasquez Aleman[1], Hanny Moreira[1], Jaime Cardona-Ospina[3], Sandra Bos[4], Guillermina Kuan[1], Federico Engel Narvaez[5], Eva Harris[3], Angel Balmaseda[1]

[1]Sustainable Sciences Institute, Managua, Nicaragua, [2]Sustainable Sciences Institute, Oakland, CA, United States, [3]Division of Infectious Diseases and Vaccinology, School of Public Health, University of California, Berkeley, Berkeley, CA, United States, [4]Deparment of Infectious Diseases and Microbiology, School of Public Health, University of Pittsburgh, Pittsburgh, PA, United States, [5]Hospital Infantil Manuel de Jesús Rivera, Ministerio de Salud, Managua, Nicaragua

The 4 dengue virus serotypes (DENV1-4) are a major global public health concern. DENV3 (lineage III.C.2.1) has historically been associated with clinical severity in Nicaragua. After no circulation since 2015, DENV3 re-emerged in 2022 with a new lineage (III.B.3.2). Using data from 2 longstanding pediatric dengue studies in Managua, we compared the clinical characteristics of these 2 lineages. DENV3 cases (ages 6 months-17 years) were confirmed by molecular and serological methods. Sequencing was performed using Oxford Nanopore and Illumina. DENV3 lineages were stratified per Nexstrain, enabling classification into Historical (2007-2014) and Emergent (≥2022) lineage periods, with no co-circulation detected. Immune status (primary [1°] vs secondary [2°] response) was determined by Inhibition ELISA. Outcomes included Severe Dengue [SD] (WHO 2009), DHF/DSS (WHO 1997), shock, plasma leakage (PL), and gallbladder wall thickening (GBWT)—an ultrasound marker of capillary leak not affected by fluid management. We performed a multivariable logistic regression controlling for immune status, age, and sex. Of 1,317 DENV3 cases, the III.B.3.2 lineage showed a higher prevalence in 2° infections (71.3% vs. 47.6%; OR 2.73, 95% confidence interval (CI) 2.18-3.43, p<0.001). In the multivariable model, the emergent lineage was independently associated with a reduction of GBWT (aOR 0.42, 95% CI 0.26-0.66, p<0.001) and PL (aOR 0.20, 95% CI 0.15-0.26, p<0.001), as well as 94% in the odds of SD (aOR 0.06, 95% CI 0.04-0.10, p<0.001) and shock (aOR 0.06, 95% CI 0.03-0.09, p<0.001) and 92% of DHF/DSS (aOR 0.08, 95% CI 0.05-0.14, p<0.001). Notably, the III.B.3.2 lineage showed a reduction in all severe outcomes in both 1° and 2° infections. Improvement in fluid and case management during the study period also likely contributed to reduced severity. In our study, the emergent DENV3 lineage exhibited marked phenotypic attenuation compared to the historical lineage, suggesting that lineage-specific viral factors contribute to modulating current outcomes. These findings underscore the importance of integrated continuous genomic and clinical surveillance.

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VACCINE INDUCED JAPANESE ENCEPHALITIS VIRUS ANTIBODY DYNAMICS AND THEIR RELATIONSHIP TO DENGUE INFECTION AND DISEASE RISK IN THAILAND

Marco Hamins-Puertolas[1], Ruchira Ruth Khosavanna[2], Darunee Buddhari[3], Henrik Salje[4], Angkana T. Huang[5], Taweewun Hunsawong[6], Derek Cummings[7], Stefan Fernandez[8], Aaron Farmer[9], Surachai Kaewhiran[10], Anon Srikiatkhachorn[11], Sopon Iamsirithaworn[12], Adam Waickman[13], Stephen J. Thomas[14], Timothy Paul Endy[15], Alan L. Rothman[16], Isabel Rodriguez-Barraquer[17], Kathryn B. Anderson[13]

[1]State University of New York Upstate Medical University, Syracuse, NY, United States, [2]Bridgeport, CT, United States, [3]KAVRU USAMD-AFRIMS, Phyathai, Thailand, [4]University of Cambridge, CAMBRIDGE, United Kingdom, [5]University of Cambridge, Cambridge, United Kingdom, [6]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [7]Johns Hopkins University, Baltimore, MD, United States, [8]AFRIMS, Bangkok, Thailand, [9]AFRIMS, Silver Spring, MD, United States, [10]Kamphaeng Phet Hospital, Kamphaeng Phet, Thailand, [11]University of Rhode Island, Providence, RI, United States, [12]Bureau of Epidemiology Thailand, Nonthaburi, Thailand, [13]SUNY Upstate Medical University, Syracuse, NY, United States, [14]Skaneateles, NY, United States, [15]CEPI, Bloomsbury, United Kingdom, [16]Institute for Immunology and Informatics, Framingham, MA, United States, [17]University of California, San Francisco, San Francisco, CA, United States

Multiple orthoflaviruses co-circulate in Southeast Asia including Japanese encephalitis virus (JEV) and dengue virus (DENV), leading to complex immunity developing from birth. In Thailand, JEV vaccination is widespread, often the first orthoflavivirus exposure, and leads to the development of cross-reactive antibodies that may alter DENV infection or disease risk, potentially via antibody-dependent enhancement (ADE). Here we aim to

identify if there is any impact of JEV vaccine induced cross-reactive titers on dengue infection and disease risk. We leverage serological data from over 26 years across three cohort studies based in Kamphaeng Phet, Thailand (1998-2024) where over 37,000 serological samples from 6,500 children were collected. This spanned the rollout of the inactivated JEV vaccine and the introduction of the live-attenuated JEV vaccine in Thailand, with multiple doses administered before age 3. The hemagglutination inhibition assay was used to quantify antibody titers for JEV and all four DENV serotypes, allowing for the characterization of maternal and vaccine mediated antibody kinetics. We found that maternal and newborn JEV titers were highly correlated (r = 0.91), with rapid decay in early infancy (half-life of 36.9 days) and loss of detectable antibodies within the first months of life (median time to seronegative response - 115.58 days). Following vaccination, JEV antibody decay slowed with each successive dose (half lives of 19.4, 90.9, and 115.8 after the first through third doses), indicating increased durability, while boosts increased. We evaluated the association between JEV vaccination and subsequent DENV infection or disease risk using mixed-effects, time-to-event, and Bayesian mechanistic models. No significant association was found using the two former statistical approaches, while mechanistic modeling that leveraged local hospitalization data suggested that intermediate antibody titers could act as disease risk windows, a pattern consistent with ADE. These findings highlight the complex interplay across orthoflavivirus immunity and their contributions to dengue infection and disease risk.

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RIFT VALLEY FEVER OUTBREAK IN SENEGAL, 2025

Bocar Sow[1], Abdourahmane Sow[1], Oumar Faye[1], Mawlouth Diallo[1], Aboubacry Gaye[1], Gad Papin Oholiab Namndiro[1], Moussa Moïse Diagne[1], Gamou Fall[1], Alioune Gaye[1], Abiboulaye Sall[2], Mamadou Sarr Ndao[1], Samba Niang Sagne[1], Ndeye Khady Diatou Ndiaye[1], Ndeye Awa Ndiaye[1], Marie Henriette Dior Ndione[1], El hadji Ndiaye[1], Mignane Ndiaye[1], Seynabou Mbaye Ba Souna Diop[1], Safiétou Sankhe[1], Mouhamed Kane[1], Joseph R. A. Fitchett[1], Ibrahima Dia[1], Cheikh Loucoubar[1], Ousmane Faye[1], Ndongo Dia[1], Mamadou Aliou Barry[1], Boubacar Diallo[1], Diawo Diallo[1], Boly Diop[3], Ousmane Cisse[3], Mamadou Ndiaye[3], Ibrahima Socé Fall[1]

[1]Institut Pasteur de Dakar, Dakar, Senegal, [2]Saint - Louis, SL, Senegal, [3]Ministry of Health and Public Hygiene, Dakar, Senegal

Since September 21, 2025, Senegal has experienced a large outbreak of Rift Valley fever following periods of heavy rainfall that created favorable ecological conditions for mosquito proliferation in northern pastoral areas. By December 15, 2025, a total of 553 laboratory-confirmed human cases, including 31 deaths (case-fatality rate 5.6%), had been identified among 18,428 persons tested during investigations. Most cases were reported in northern livestock-rearing regions, particularly Saint-Louis, with additional cases detected in neighboring areas. The median age of confirmed cases was 26 years (interquartile range 20-38), 65.1% were male, and more than half were 15-30 years old, reflecting high exposure among young adults involved in livestock activities. Clinical manifestations ranged from mild febrile illness to severe complications, with hemorrhagic or neurologic signs observed in approximately 9.2% of confirmed patients. Entomological investigations conducted in affected localities collected 14,009 mosquitoes, of which Culex tritaeniorhynchus represented 81.6% of captures; 16 mosquito pools tested positive for Rift Valley fever virus by molecular assays. Genomic sequencing of approximately 45 human samples, together with viral isolates obtained from livestock and mosquito vectors, revealed circulation of lineage H across the vector-ruminant-human transmission cycle without mutations clearly associated with increased clinical severity. Public health response measures included intensified human and animal surveillance, vaccination of susceptible ruminant populations, targeted vector control interventions, and risk communication in affected communities. These findings highlight the continued circulation of Rift Valley fever virus in northern Senegal and underscore the importance of integrated One Health surveillance, early outbreak detection, and rapid response strategies to mitigate the impact of climate-sensitive zoonotic arboviruses in West Africa.

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THE EFFECT OF PRIOR ZIKA AND DENGUE EXPOSURE ON THE RISK OF SEROTYPE-SPECIFIC DENGUE DISEASE

Cassia F. Estofolete[1], **Gabriel Ribeiro dos Santos**[2], Bruno Milhin[1], Juan Borrego[3], Matt D.T. Hitchings[4], Derek A.T. Cummings[2], Albert Icksang Ko[3], Mauricio L. Nogueira[1]

[1]Faculdade de Medicina de Sao Jose Do Rio Preto, SJ Rio Preto, Brazil, [2]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [3]Yale School of Public Health, New Haven, CT, United States, [4]University of Florida, Gainesville, FL, United States

Prior flavivirus exposure may increase dengue severity through antibody-dependent enhancement (ADE) or decrease it through cross-protection. Using a test-negative design of patients who presented to an urgent care facility of São José do Rio Preto, Brazil, we evaluated the association between IgG titers for Zika virus (ZIKV) and Dengue virus (DENV) at the time of identification and subsequent clinical outcomes due to DENV1 and DENV2 infection. We enrolled patients presenting with dengue-like illness from 2019 to 2022, spanning a DENV2 outbreak and two DENV1 outbreaks. PCR testing was used to identify cases of DENV infection. Prior exposure to DENV and ZIKV was assessed at the time of presentation by IgG ELISA. Optical Density (OD) ratio was used as a proxy for IgG concentration. A regression framework adjusting for confounders was fitted to estimate the variation in odds of clinical dengue when being seropositive to ZIKV only, to DENV only or to both compared to being negative to both. A second model included OD ratios as a continuous variable. Among 4,026 participants, 790 were PCR-positive for DENV1 and 348 for DENV2. Seropositivity to ZIKV only was associated with lower odds of DENV1 (aOR 0.34, 95%CI 0.14-0.82) but higher odds of DENV2 (aOR 6.62, 95%CI 2.50-17.56). Seropositivity to DENV only and to both ZIKV and DENV were associated with reduced odds of dengue for both serotypes. For DENV1, risk followed a non-linear pattern with DENV IgG: odds peaked at intermediate titers (max aOR 1.73, 95%CI 1.18-2.40 at OD 0.86). Our findings indicate that the effect of prior ZIKV exposure on dengue outcomes is serotype-specific where the risk for clinical dengue is increased for DENV2 infection and reduced for DENV1 infection. This strengthens findings in prior studies. The observed increased risk of dengue at intermediate levels of DENV IgG supports ADE-consistent dynamics and suggests ELISA OD ratios may help identify individuals at risk of disease. These findings highlight the relevance of incorporating ZIKV serology into dengue vaccine design and implementation, as ZIKV immunity may differentially shape serotype-specific vaccine performance and safety.

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POPULATION GENOMICS REVEALS MULTI-SCALE MECHANISMS SUSTAINING SCHISTOSOMIASIS RE-EMERGENCE IN A NEAR-ELIMINATION SETTING

Hannah Diane Guss[1], Yannick Francioli[1], Elise N. Grover[2], Andrew Hill[2], Wei Zou[2], Kristen Wade[3], Hamish Pike[4], Siddharth Gopalan[1], Liu Yang[5], Zhong Bo[5], David Pollock[2], Elizabeth Carlton[6], Todd Castoe[1]

[1]University of Texas at Arlington, Arlington, TX, United States, [2]University of Colorado Anschutz Medical Campus, Aurora, CO, United States, [3]University of California San Francisco, San Francisco, CA, United States, [4]Weizmann Institute of Science, Rehovot, Israel, [5]Sichuan Center for Disease Control and Prevention, Chengdu, China, [6]University of Colorado, Aurora, CO, United States

Despite decades of intensive control, schistosomiasis re-emerged in Sichuan Province, China following near-elimination, highlighting critical gaps in our understanding of transmission dynamics in late-stage control settings. Here, we present a population genomic investigation of *Schistosoma japonicum* re-emergence, leveraging whole-genome sequences from 270 miracidia collected from 53 human hosts across 17 villages in 2007, immediately following resurgence. Genome-wide analyses revealed a largely cohesive regional parasite population with weak geographic structure and no evidence of recent effective population size decline, suggesting persistence of standing genetic diversity despite prolonged control. At finer

spatial scales, several villages exhibited reduced diversity and elevated inbreeding, consistent with localized transmission maintained by small founding populations. Pairwise relatedness analyses identified dense within-host and within-village sibling clusters alongside second- and third-degree relationships spanning villages, and rare but informative close-kin links between villages. These patterns support a model of highly local transmission embedded within a broader, connected transmission network with episodic parasite movement. Estimated minimum worm burdens varied widely among hosts, further indicating heterogeneity in infection complexity. Together, these findings suggest that re-emergence in this near-elimination setting was facilitated by a genetically diverse parasite population likely maintained in non-human mammalian hosts, coupled with predominantly local transmission and occasional inter-village connectivity. This study demonstrates the power of population genomics for genetic surveillance in near-elimination contexts, providing a high-resolution framework to identify transmission pathways and hidden reservoirs that may undermine control efforts in complex, multi-host systems.

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ASSOCIATION BETWEEN SERUM BIOMARKERS AND ULTRASONOGRAPHIC IMAGE PATTERNS OF LIVER FIBROSIS IN A *SCHISTOSOMA JAPONICUM* ENDEMIC AREA IN THE PHILIPPINES

Ralph Narido Aniceto[1], Kanika Men[2], Sangshin Park[3], **Hannah Wu**[4], Joachim Richter[5], Jonathan D. Kurtis, MD/PhD[6], Mario Jiz[7], Jennifer F. Friedman[8]

[1]Research Institute for Tropical Medicine, Tacloban, Philippines, [2]Center for International Health Research, Brown University, School of Medicine, Providence, RI, United States, [3]University of Seoul, Seoul, Korea, Democratic People's Republic of, [4]Brown University, School of Medicine, Providence, RI, United States, [5]Institute of International Health, Charité Centre for Global Health, Charité University Medicine, Free University and Humboldt University, Berlin, Germany, [6]Brown University Medical School, Providence, RI, United States, [7]Research Institute for Tropical Medicine, Muntinlupa City, Philippines, [8]Center for International Health Research, Providence, RI, United States

Schistosomiasis-associated liver injury is driven by granulomatous inflammation, resulting in excessive extracellular matrix deposition and progressive fibrosis. The relationship between circulating biomarkers, including those reflecting both fibroproliferation and fibrodegradation, and clinical fibrosis status remains poorly defined. This study evaluated selected serum biomarkers alongside an indirect fibrosis index, Aspartate Aminotransferase(AST)-to-Platelet Ratio Index (APRI) to characterize schistosomiasis-specific liver pathology defined by ultrasound image patterns (IP) using the Basel Protocol. We enrolled 420 participants from an *S. japonicum*-endemic area in Jaro, Leyte, Philippines. Serum levels of MMP-1, MMP-2, MMP-9, TIMP-1, TIMP-3, PICP, and IGFBP-3 were quantified using a multiplex bead-based assay. APRI (Aspartate Aminotransferase-to-Platelet Ratio Index) was calculated from concurrent hematologic data. Data were log-transformed to ensure normality. Pearson correlation and multivariate logistic regression were used to assess associations and estimate odds ratios (OR) across IP groups: interseptal fibrosis (ISF), periportal fibrosis (PF), and mixed (PF+ISF). Correlation analysis demonstrated a coordinated protease response, with MMP-2 strongly correlated with MMP-9 ($r=0.350$, $p<0.0001$, $n=326$) and MMP-1 ($r=0.346$, $p<0.0001$, $n=326$). Multivariate analysis showed that participants with the mixed IP had a markedly higher likelihood of elevated APRI (OR 17.33, 95% CI 2.96-101.41), whereas no significant associations were observed for ISF or PF alone. When APRI was categorized into tertiles, values >0.288 were associated with an increased likelihood of mixed IP (OR 3.10, 95% CI 1.16-8.33). Higher MMP-9 was associated with a reduced risk of ISF (OR 0.25, 95% CI 0.07-0.92), suggesting this multifunctional protease has a fibrodegradative effect that helps prevent this specific fibrosis pattern in *S. japonicum* infection. These findings indicate that APRI and MMP-9 may serve as complementary biosignatures for monitoring disease progression in schistosomiasis-associated liver fibrosis.

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THE DEOPRHANIZATION OF A NEW DOPAMINE RECEPTOR IN *SCHISTOSOMA MANSONI*

M. Evelyn Carpenter, Erika Bistran, **Daniel J. Sprague**

Medical University of South Carolina, Charleston, SC, United States

Schistosomes form a genus of parasitic flatworms that cause schistosomiasis, a global disease of severe morbidity and mortality. Schistosomiasis affects more than 250 million people and places almost 1 billion people at risk in endemic regions. Chronic infection results in the deposition of parasite eggs within host tissues, triggering a sustained inflammatory response. Over time, this immune reaction leads to granuloma formation and progressive fibrosis, the primary drivers of pathology in infected individuals. Praziquantel is the only FDA-approved treatment, and emerging reports of reduced efficacy highlight an urgent need for alternative therapeutic strategies. To address this, and to better understand parasite physiology, we have taken a systematic approach of deorphanizing the GPCRome of schistosomes and related parasitic flatworms. G protein coupled receptors (GPCRs) are highly druggable, evidenced by the fact that they are the largest class of receptors that are targeted by FDA-approved drugs. However, to date, >90% of the putative GPCRs in schistosomes have remained unstudied, and the few that have been studied only have sparse reports in the literature. Here, we report the characterization of a previously unreported dopamine receptor from *Schistosoma mansoni*, and related worms. Using a real-time cAMP biosensor assay, we demonstrate that this receptor selectively responds to dopamine over other common bioaminergic ligands. Computational modeling predicts this receptor to be a canonical Class A GPCR, and using mutational studies, we demonstrate key residues that are critical for activation of the receptor by different ligands. Finally, a preliminary screen of ~3,000 small molecules identified key agonists and antagonists of this receptor, including some FDA-approved ligands that may be repurposed for treating neglected tropical diseases. These findings establish the new dopamine receptor as a potential drug target, and also demonstrate the value in deorphanizing key signaling pathways in the study of neglected tropical diseases.

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CASPASE-DEPENDENT APOPTOSIS MEDIATES SCHISTOSOME DEATH INDUCED BY NOVEL THIOREDOXIN GLUTATHIONE REDUCTASE INHIBITORS

Sammy Y. Aboagye[1], Valentina Z. Petukhova[2], Luke N. Harding[2], Francesco Y. Angelucci[3], Pavel A. Petukhov[2], David L. Williams[1]

[1]Rush University Medical Center, Chicago, IL, United States, [2]University of Illinois Chicago, Chicago, IL, United States, [3]University of L'Aquila, L'Aquila, Italy

Schistosomiasis remains a major neglected tropical disease affecting over 200 million people worldwide, with chemotherapy still largely reliant on praziquantel. Although effective against adult worms, praziquantel shows limited efficacy against juvenile parasites, underscoring the urgent need for new therapeutics with improved stage-specific activity. In a recent study, we identified a series of noncovalent inhibitors targeting thioredoxin glutathione reductase (TGR), an essential enzyme that maintains redox homeostasis in schistosomes. These compounds demonstrate robust in vivo efficacy in murine models, meet World Health Organization criteria for lead progression, and exhibit superior activity against juvenile worms compared to praziquantel. Here, we investigated the mechanism underlying parasite killing induced by these TGR inhibitors, specifically assessing whether compound-mediated death occurs through caspase-dependent apoptosis or alternative non-apoptotic pathways. Using luminescence-based assays in both adult worms and schistosomula, treatment with TGR inhibitors consistently induced a marked increase in luminescence signals, indicative of caspase-dependent apoptotic activity. This effect was reproducible across multiple compounds, suggesting a shared mechanism of action. To establish pathway specificity, parasites were co-treated with the pan-caspase inhibitor Z-VAD(OMe)-FMK. Co-treatment significantly attenuated luminescence signals and partially rescued parasite viability, indicating

that caspase activation is a principal driver of compound-induced death. In contrast, inhibition of non-apoptotic pathways, including ferroptosis-associated processes, produced only modest effects on luminescence and parasite survival. Overall, our findings demonstrate that noncovalent TGR inhibitors induce schistosome death predominantly through caspase-dependent apoptotic mechanisms, supporting their development as next-generation antischistosomal therapeutics.

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PROCESS DEVELOPMENT AND EFFICACY TESTING OF *SCHISTOSOMA HAEMATOBIUM* CALPAIN-BASED NEXT-GENERATION VACCINES FOR UROGENITAL SCHISTOSOMIASIS

Deborah O. Molehin[1], Christine N. Lee[1], Shelby McMurray[1], Shannan Ganpat[1], Hollie Chiles[1], Eunice Choi[1], Jenn Davis[2], Kim Jiho[2], Darrick Carter[2], Sean Alex Gray[2], **Adebayo James Molehin**[1]

[1]Midwestern University, Glendale, AZ, United States, [2]PAI Life Sciences, Inc., Seattle, WA, United States

Schistosomiasis, a neglected tropical disease caused by parasitic helminths, *Schistosoma* spp, affects ~400-600 million people globally with an additional 800 million people at risk of infection. *S. haematobium* is the causative agent of urogenital schistosomiasis (UGS), a chronic disease characterized by hematuria, genital lesions, bladder cancer and increased susceptibility to sexually transmitted infections. Reports of transmission in Europe highlight the disease's expanding reach. Current control efforts rely on mass drug administration of praziquantel which targets adult worms but not juveniles, does not prevent reinfection, and sustained use raises concern for drug resistance. An effective vaccine is therefore essential for sustainable control and elimination. Building on our prior success with *S. mansoni* Sm-p80 vaccine (SchistoShield®), now in clinical trials, we assessed the efficacy of the *S. haematobium* ortholog Sh-p80 and a next-generation catalysis-targeted derivative, CaTaCo-Hem™ (Catalysis-Targeted Construct-haematobium). Antigens were formulated with a proprietary TLR4-based adjuvants, EmT4™ or LiT4Q™ and tested in a hamster model of UGS. Recombinant CaTaCo-Hem™ was purified as a stable monomer with reduced degradation and aggregation under non-denaturing conditions and demonstrated improved catalytic efficiency relative to full-length Sh-p80. Vaccination with CaTaCo-Hem™ induced significantly higher specific total IgG responses and stronger Th1-associated cytokine production following immunization and cercarial challenge compared with full-length Sh-p80. Both Sh-p80 and CaTaCo-Hem™ vaccination resulted in significant protection, reducing adult worm burden by 51-52% and tissue egg burden by 45-52%, with concomitant reductions in egg viability compared with controls. These findings demonstrate that catalysis-targeted calpain constructs elicit robust protective immunity against *S. haematobium* infection and support continued development of CaTaCo-Hem™ as a next-generation vaccine candidate for long-term control and elimination of urogenital schistosomiasis

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EVALUATION OF A RAPID, SIMPLE, AND AFFORDABLE NUCLEIC ACID AMPLIFICATION TEST FOR *VIBRIO CHOLERA, CAMPYLOBACTER JEJUNI/COLI,* AND *SHIGELLA* SPP.

Kelly K. Baker[1], Jillian McGee[2], Ellie Madson[1], Abu S.G. Faruque[3], Farina Naz[4], Mosabbir Ahmed[4], Asaduzzaman Asad[4], Zhahirul Islam[4], John Agira[5], Sheillah Simiyu[6], Haekyung Shin[7], Xiao Jiang[7], Chang Hee Kim[8]

[1]State University of New York at Buffalo, Buffalo, NY, United States, [2]GoDx, Madison, WI, United States, [3]icddr,b, Dhaka, United States, [4]icddrb, Dhaka, Bangladesh, [5]African Population Health Research Center, Nairobi, Kenya, [6]Nairobi, Kenya, [7]GoDiagnostic, Madison, WI, United States, [8]Madison, WI, United States

Vibrio cholerae, Shigella spp., and *Campylobacter jejuni/coli* diarrheal infections pose a severe risk of disease and are recommended for empiric antibiotic treatment under World Health Organization guidelines. The complex etiology of diarrhea can lead to syndromic misdiagnosis of cases and unnecessary antibiotic use. Access to simple, affordable, rapid point-of-care tests could improve the accuracy of diagnosis and speed and appropriateness of antibiotic treatment decisions. This study evaluated a prototype paper strip diagnostic assay using isothermal DNA amplification and a heat block to target known bacterial pathogenesis genes with lateral flow assay readout. The limit of detection and reproducibility of each assay in controlled conditions was tested using DNA spiked in water and feces for each assay, alone and in combination. *Campylobacter, Shigella*, and V. *cholerae* assays detected 20 of 21 replicates at $2x10^1$, $2x10^3$, and $2x10^2$ cfu/mg feces, respectively, with no evidence of false positives or cross-reactivity. Field evaluation used de-identified culture and molecular test-positive and negative feces specimens collected in Kenya and Bangladesh for a technician-blinded test of READI assays for *V. cholerae* (n=216)*, C. jejuni/coli* (n=142), and *Shigella* spp. (n=191). The *V. cholerae* assay achieved fair accuracy compared to qPCR (75.9%) and culture (78.7%). Accuracy of READI *C. jejuni/coli* was poor (<60%) compared to qPCR but fair compared to culture (76.4%). Accuracy of READI *Shigella* was poor (<60%) compared to qPCR but good (84%) compared to culture. Interuser disagreement on READI results (adjusted OR (adjOR)=5.34, 95% CI=2.12, 13.46) and minutes spent running batch assays (adjOR=0.99, 95% CI=0.988, 0.999) explained discordance with qPCR results. Interuser disagreement on READI results (adjOR=7.60, 95% CI=2.37, 24.32) and increases in ambient humidity (adjOR=1.06, 95% CI=1.01, 1.11) explained discordance with culture results. The assay evaluated represents a prototype under active development, and findings from this study are informing ongoing optimization of the platform and protocols for use.

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DIGITAL REPORTING AND DOCUMENTATION ACCURACY IN AZITHROMYCIN MASS DRUG ADMINISTRATION: DOES EXPERIENCE FROM TWO ROUNDS IMPROVE PERFORMANCE?

Hassan Seun Ogunwemimo, Adaeze Catherine Aidenagbon, Abdulmalik Alilu Abubakar, Abdulmajid Safana Idris, Obiora Anthony Anidebe, Yusuf Saje Yakubu, Hafsat Baba Musa, Emmanuel Eneojo Akagu

Malaria Consortium, Abuja, Nigeria

Azithromycin mass drug administration (AZM MDA) for children 1-59 months is the core intervention of the SARMAAN II project in Nigeria. Two rounds during 2025-2026 deployed DIGIT Health Campaign Management (HCM) and the NAFDAC MedSafety Application across three Nigerian states, replacing paper-based systems often affected by logistical and documentation challenges, to improve efficiency, accountability, and real-time problem-solving in AZM MDA. We probed whether Round 1 experience predicted accurate Round 2 digital reporting and documentation. A cross-sectional analysis of 6,758 supervision records from two campaign rounds was conducted in Jigawa, Kaduna and Kebbi States. Six digital reporting metrics were assessed: Officer-in-Charge (OIC) device provisioning; DIGIT HCM-documentation of AZM issued; DIGIT HCM-documentation of AZM returned; community drug distributors (CDD) device provisioning; CDD DIGIT HCM use; and the NAFDAC application on OIC devices. Issue and Receive Voucher (IRV) availability; distribution plan-IRV concordance; and pharmacovigilance (PV) forms completeness were accuracy measures. Chi-square tests assessed differences between rounds, and multivariable logistic regression models adjusted for round, state and facility type. Digital reporting was consistently high (≥ 97%; range: 97.3-99.6%). No indicators improved significantly between rounds (all $p>0.05$). Round 2 IRV availability fell to 99.2% from 99.6% in Round 1 ($\chi^2=4.35$, $p=0.037$; aOR=0.45). OIC return documentation was significantly lower in Kaduna (aOR=0.47, $p=0.002$) and Kebbi (aOR=0.38, $p<0.001$) compared to Jigawa. PV accuracy differed by state: Jigawa 97.8%, Kaduna 75.4%, and Kebbi 74.6%, all had lower adjusted odds (aOR=0.08, $p<0.001$). Digital reporting remained nearly universal across both rounds. However, prior campaign experience did not inform measurable improvements in documentation accuracy, and state level differences

persisted. We recommend strengthening PV training in Kaduna and Kebbi, improving IRV supply chains in Kebbi, addressing gaps in the NAFDAC application, and providing targeted CDD digital task support.

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INFORMATICS-ASSISTED DIAGNOSIS OF INFECTIOUS DISEASES: BEYOND CLINICAL INTUITION

Yael Berger, **Jean-Felipe Teotonio**, Yaakov Dickstein, Jonathan B. Gubbay, Varsha Reddy, Stephen Berger

Gideon Informatics Inc, Los Angeles, CA, United States

The Case Records of the Massachusetts General Hospital (MGH) present expert clinical reasoning in cases of diagnostic complexity. This study evaluates the utility of a commercial expert system for resolving challenging infectious disease cases through Bayesian analysis. We retrospectively analyzed New England Journal of Medicine (NEJM) Infectious Disease MGH Case Records (2021-2025). Polymicrobial infections, non-infectious diseases that mimic infection, and cases lacking contemporary diagnostic standards were excluded. Clinical data, including patient demographics and medical history, were extracted, and the Global Infectious Diseases and Epidemiology Online Network (GIDEON) diagnostic module was used to generate a ranked differential diagnosis list. Diagnostic utility was assessed via a three-stage sequential analysis: (1) baseline clinical findings; (2) exclusion of infections ruled out as described in each case report; and (3) post-hoc integration of specific vector exposures. Forty-five case records were analyzed (mean age 46 years; 56% male). Most cases (87%) were acquired in North America, with 11% related to immigration or travel. The correct diagnosis was included in the differential diagnosis list in 98% (n=44) of cases, with a median of seven diseases per list. Accuracy improved significantly ($p < .05$) across the stages of analysis: the NEJM diagnosis was ranked as one of the top two differentials in 71% (n=32) of cases at baseline (49% and 22% as first and second, respectively); increasing to 80% (n=36) after exclusions (49% and 31% as first and second, respectively); and reaching 89% (n=40) on post-hoc integration of vector exposure data (67% and 22% as first and second, respectively). In conclusion, informatics-assisted tools demonstrate a high diagnostic utility through mirroring expert reasoning to resolve complex infectious disease cases, particularly when incorporating epidemiological exposure information. Larger prospective studies are warranted to further validate these systems in diverse clinical scenarios.

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DEVELOPING AN AI-POWERED INTERFACE FOR A MALARIA EPIDEMIOLOGY AND GENOMICS DASHBOARD TO SUPPORT PROGRAMMATIC DECISION-MAKING

Katherine E. Battle[1], Bassirou Ngom[2], David Kong[1], Cherif Diouf[2], Amy Gaye[2], Djiby Sow[2], Mamadou A. Diallo[2], Mariama Toure[2], Alejandro Echeverria[1], Emily K. Driano[1], Dejan Lukacevic[1], Jennifer Simeon[1], Elhadji Doucoure[3], Medoune Ndiop[4], Ibrahima Diallo[3], Aliou Thiongane[3], Dyann F. Wirth[5], Caitlin Bever[1], Sarah K. Volkman[5], Daouda Ndiaye[2]

[1]Institute for Disease Modeling, Gates Foundation, Seattle, WA, United States, [2]Centre International de Recherche et de Formation en Génomique Appliquée et de la Surveillance Sanitaire, Dakar, Senegal, [3]National Malaria Control Program (NMCP), Senegal, Dakar, Senegal, [4]NMCP Senegal, Dakar, Senegal, [5]Harvard T.H. Chan School of Public Health, Broad Institute, Boston, MA, United States

National malaria control programs (NMCPs) require timely access to diverse, integrated data to guide effective programmatic decisions. In Senegal, the NMCP has partnered with the Institute for Disease Modeling (IDM, Gates Foundation) and the Centre International de Recherche et de Formation en Génomique Appliquée et de Surveillance Sanitaire (CIGASS) at the Université Cheikh Anta Diop (UCAD) to develop a dashboard integrating a broad suite of indicators from multiple institutions and data sources. These include routine surveillance indicators covering incidence and intervention coverage, alongside genomic surveillance data tracking drug resistance markers, pfhrp2/3 gene deletions, and genetic diversity metrics.While the dashboard has demonstrated utility for data integration and visualization, extracting actionable insights from this breadth of data remains challenging. To address this, we developed an AI-powered chat interface enabling users to query dashboard data and previous NMCP reports using natural language. Leveraging large language models, the tool produces summarized, contextualized, and actionable responses that would be prohibitively time-consuming to generate manually. For example, users can rapidly identify areas where incidence trends deviate from expectations given intervention coverage, or where drug resistance markers overlap with interventions such as seasonal malaria chemoprevention. We are also developing an AI-powered companion tool to simplify building similar dashboards and incorporating new data sources. This will lower the technical barrier for NMCPs to establish their own integrated surveillance platforms.We present the design of these AI tools, demonstrate their application using examples from Senegal, and discuss their potential to strengthen data-driven decision-making for malaria control programs.

6065

STRENGTHENING SUSTAINABLE SYNDROMIC AND VECTOR SURVEILLANCE AT POINTS OF ENTRY IN GHANA

Linda Boatemaa

Noguchi Memorial Institute for Medical Research, Accra, Ghana

Points of Entry (PoEs) are critical interfaces for the introduction and spread of vector-borne pathogens due to high volumes of human mobility and trade. Strengthening vector surveillance at PoEs is therefore essential for early warning and preparedness against emerging and re-emerging infectious diseases. This study aimed to strengthen sustainable mosquito-based surveillance at selected PoEs in Ghana, with limited exploratory sampling of small mammal reservoirs. Mosquito surveillance was conducted at Tema Port and Aflao Border between September and November 2024 following standardized entomological protocols. Adult mosquitoes were collected, morphologically identified, pooled by species, and screened using molecular assays for priority arboviruses including dengue, Zika, chikungunya, yellow fever, West Nile, and Rift Valley fever viruses. As a supplementary component, a small number of rodents were opportunistically sampled to explore the feasibility of integrated vector-reservoir surveillance. A total of 12,579 mosquitoes, predominantly *Aedes* and *Culex* species, were collected across both PoEs and screened for arboviral pathogens. All mosquito pools tested negative for the targeted viruses. Eight rodents were captured during the surveillance period and similarly tested negative for the screened zoonotic pathogens. No symptomatic travelers were identified during the study period. In conclusion, Although no active circulation of priority arboviruses was detected during the surveillance period, this mosquito-focused PoE surveillance activity demonstrates the feasibility and value of sustained entomological monitoring supported by international collaboration. Continued multi-season mosquito surveillance, integrated with laboratory capacity strengthening, is essential to enhance early detection and preparedness for vector-borne disease threats at Ghana's Points of Entry.

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TRANSFORMING MALARIA PREVENTION LOGISTICS IN MALI: DIGITAL STOCK MANAGEMENT FOR SMC

Boubacar Sidiki Maiga[1], Cheikh Gassama[1], KEITA Fatoumata[1], Alassane Bangoura[1], Kamate Jean Baptiste[1], Suzanne Van Hulle[2], Yameni Chrestien[3], Mady Cissoko[4], Aissata Kone[4]

[1]Catholic Relief Services - CRS, Bamako, Mali, [2]Catholic Relief Services, Baltimore, MD, United States, [3]Catholic Relief Services, Dakar, Senegal, [4]National Malaria Control Program - Mali, Bamako, Mali

Seasonal Malaria Chemoprevention (SMCP) in Mali previously relied on paper-based systems, which limited visibility, responsiveness, and accountability. This led to frequent stockouts and poor traceability of medications. To address these challenges, in 2025, a digital inventory management system was deployed in 16 health districts, covering 4,920 community health workers and technical center directors, as well as 372

health facilities. This digitization process included two mobile modules: daily reconciliation of medications administered by community health workers on a day-to-day basis during the campaign, and end-of-campaign inventory counts at health facilities conducted by center directors. Inventory data (receipts, distribution, consumption, and remaining stock) were entered in real time and synchronized in DHIS2, enabling centralized monitoring, triangulation, and dynamic analysis. The performance metrics analyzed included the completeness and timeliness of reports, inventory discrepancies, stockouts, and the traceability of medications through batch number tracking. After digitization, the completeness and timeliness of reports exceeded 90%, representing a major improvement over previous campaigns. Inventory discrepancies decreased by 75%, while stockouts were reduced by more than 95%, demonstrating the system's effectiveness in proactive product management. Full traceability has strengthened accountability at all levels, and integration with existing oversight procedures has yielded measurable efficiency gains, including a reduction in administrative burden and operational costs. This study demonstrates that an integrated digital approach can transform the performance of the CPS supply chain in resource-constrained settings. By combining real-time visibility, traceability, and operational efficiency, this model provides a scalable and replicable solution for optimizing large-scale malaria control campaigns.

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HUMAN AND AI WORKING HAND-IN-HAND TO IMPROVE DIGITAL MICROSCOPY OF PARASITIC INFECTIONS

Skyler Colwell, Vanessa D'Amario, Ryan Vassalotti, Jorge Cervantes

Nova Southeastern Univeristy, Davie, FL, United States

Microscopy is the gold standard for diagnosing many tropical infections. Digital microscopy is being revolutionized by AI, but model training requires quality labeled datasets. Manually labeling large datasets for development of supervised machine learning models for point of care diagnostics is often limited by human time and expertise. In order to recognize areas of a digital image a machine learning model may fixate on, we built an open source semi-automated annotation pipeline that identifies regions of interest (bounding boxes) on parasite slide images. Our detector uses five distinct detection strategies to automatically identify bounding boxes: color segmentation for stained specimens, local contrast subtraction, edge density, dark object thresholding, and a z-score anomaly detector for faint objects. It auto-detects the circular Field of View, normalizes illumination via Contrast Limited Adaptive Histogram Equalization, and applies non-maximum suppression to merge overlapping proposals. Our pipeline allowed for human annotators to evaluate the identified bounding boxes. Annotators can rapidly edit, delete, or draw new annotations on these identified bounding boxes. Final annotations can then be exported in standard AI-interpretable formats for future model training. We evaluated the pipeline on digital images of parasitic ova captured using smart phone attachable microscopes. Our review tool was faster (18 seconds per image) compared to drawing boxes by hand (90 seconds per image). This pipeline was able to consistently recognize the same boxes regardless of the user. This work will allow for labeling a dataset for creation of AI based, smartphone acquired digital image analysis. This will bypass the requirement for expensive microscopes and software for traditional imagining analysis, making it a practical option for low-resource settings

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INTEGRATION OF VECTORCAM INTO UGANDA'S VECTOR SURVEILLANCE PROGRAM : EXPERIENCE FROM IMPLEMENTING CO-DESIGNED IMPLEMENTATION STRATEGIES

Marina Rincon Torroella[1], Samara Toussaint[1], Sunny Patel[1], Aryaman Shodhan[2], Kigongo Siriman[3], Mei-Li Hey[1], Patience Oputan[4], Justine Inhensiko[4], Ronal Wasswa[4], Peter Waiswa[4], Winnie Kansiime[4], Jimmy Opigo[3], Alex Musiime[3], James Kaweesa[5], Jovan Batte[3], David Onanyang[3], Neil Lobo[6], Catherine Maiteki-Sebuguzi[3], Youseph Yazdi[1], Diwakar Mohan[1], Soumyadipta Acharya[1]

[1]Johns Hopkins University, Baltimore, MD, United States, [2]Baltimore, MD, United States, [3]Ministry of Health, Kampala, Uganda, [4]Makerere Univeristy, Kampala, Uganda, [5]Ministry of Health, Kampal, Uganda, [6]Univ of Notre Dame, South Bend, IN, United States

Globally, 282 million cases of malaria were reported in 2024, with 95% located in Africa. Digital tools utilizing artificial intelligence have the potential to improve malaria surveillance, but their effectiveness depends on robust implementation within health systems.VectorCam is an AI-enabled mosquito identification tool designed for use by community-based health workers. We present findings from a pilot implementation of an intervention package co-designed with policy makers, and stakeholders. This study is part of a mixed-methods Hybrid Type III implementation-effectiveness trial across 14 high-burden Ugandan districts, initiated with pilot implementation in Koboko and Mayuge. VectorCam was deployed through Village Health Teams, supervised by Vector Control Officers, with data integrated into the DHIS2 platform. The implementation package was co-designed using a human-centered design approach and we conducted pre- and post-training assessments to refine VectorCam's training toolkit, implemented district readiness assessments, and piloted key informant interview guides. Findings informed iterative adaptations to implementation strategies and research tools for subsequent rollout phases, with the goal of supporting scalable integration into Uganda's national surveillance systems. Initial findings demonstrate knowledge gains following training, with mean scores increasing from 64% to 78% in Koboko and 51% to 80% in Mayuge. District readiness assessments indicate moderate preparedness: 63.1% and 66.7% respectively indicators rating "good," suggesting partial system readiness. Key limitations include inconsistent access to consumables, technology, and power, which may constrain fidelity. Key informant interviews captured data on acceptability and feasibility and were refined based on the pilot findings. These results suggest that VectorCam can enhance surveillance efficiency and data quality in a task-shifting model when supported by appropriate implementation strategies. Findings highlight the importance of adaptive, learning-oriented approaches to scaling digital health tools.

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APPLICATION OF TAQMAN ARRAY CARDS FOR EVALUATING WASTEWATER EXTRACTION METHODS FOR MULTI-PATHOGEN DETECTION

Md Ohedul Islam[1], Md Ashiqul Alam Khan[1], Mostafizur Rahman[2], Abdullah Al Miraz[2], Fariya Aboni[2], Sabrina Karim Resha[2], Rashidul Haque[2], Mami Taniuchi[1]

[1]University of Virginia, Charlottesville, VA, United States, [2]Infectious Diseases Division, icddr,b, Dhaka, Bangladesh

Comprehensive infectious disease surveillance requires simultaneous detection of multiple pathogens. Molecular methods such as multiplex quantitative polymerase chain reaction (qPCR) typically detect only a small number of targets (often ≤5), making multi-disease surveillance time- and labor-intensive. TaqMan Array Cards (TAC) are 384-well microfluidic real-time qPCR platforms which can test for 47 or more pathogen targets from a single sample in one run. Due to their modular design, target assays can be added or removed to create custom panels to address unique needs of different multi-disease surveillance. We developed a custom TAC panel

targeting enteric and respiratory pathogens (22 bacteria, 24 viruses, and 2 protozoa). The card has 48 wells per sample (8 samples per card), with single plex or duplex qPCR assays in each well (1.5 µL reaction volume per well). Synthetic positive controls were used to validate the assays, and the limit of detection (LOD) was determined using standard curves. TAC was then utilized to evaluate nucleic acid extraction methods for wastewater surveillance. Wastewater samples (n = 10) were collected in Dhaka, and two separate protocols (A and B) were followed for nucleic acid extraction. Further, nucleic acids were tested using TAC to quantify the pathogens present. The LOD for each TAC assay was 100 gene copies per reaction, comparable to larger volume multiplex qPCR. Differences in the detection rate of a variety of pathogens were observed between the samples from two extraction methods. Method B showed higher detection of bacteria [72.2% (13/18)] and viral targets [31.2% (8/24)]. Method B also presented significantly higher pathogen concentrations for most bacterial and viral targets (Wilcoxon signed-rank test, $p < 0.05$). TAC provided a high throughput platform for simultaneous detection of multiple pathogens and identified a robust workflow for multi-diseases wastewater surveillance. The modular architecture of TAC offers flexibility to adapt to different surveillance priorities ranging from routine surveillance to targeted detection of geo-specific priority pathogens and emerging infections.

6070

UNDERSTANDING THE HETEROGENEITY IN CONVENTIONAL AND RAPID CHOLERA DIAGNOSTIC TEST PERFORMANCE WITH BAYESIAN LATENT CLASS MODELS

Sonia Hegde[1], Md. Asif Ahsan[2], Md Taufiqur Rahman Bhuiyan[3], Javier Perez-Saez[4], Imrul Kayes Nabil[2], Md. Naiem Hossain[2], Zahid Hasan Khan[2], Molibul Hassan Afrad Moon[5], Tahira Ahmed Rashmi[2], Mohammad Ashraful Amin[6], Md. Taufiqul Islam[3], Ashraful I. Khan[3], Firdausi Qadri[7], Andrew Azman[8]

[1]Johns Hopkins University, Baltimore, MD, United States, [2]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [3]International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Dhaka, Bangladesh, [4]University of Geneva, Geneva, Switzerland, [5]icddr,b, Dhaka, 13, Bangladesh, [6]ICDDRB, Dhaka, Bangladesh, [7]International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Mohakhali, Dhaka, Bangladesh, [8]Johns Hopkins School of Public Health, Baltimore, MD, United States

Accurate diagnosis of *V. cholera* infection is critical for the control and elimination of cholera. While microbiological culture is the gold standard for diagnosing cholera, rapid diagnostic tests (RDTs) are increasingly being used for surveillance and outbreak detection. Estimates of sensitivity and specificity of different tests, however, have varied widely across studies due to differing sampling, sample preparation methods, reference assays, and geographic contexts. To better understand how different cholera diagnostics compare to one another and how different factors influence test performance, we aimed to collect and test stool samples under optimized conditions and employ a Bayesian latent-class model that incorporates conditional dependence and key covariates to estimate sensitivity and specificity. From February 2024 to August 2025, we enrolled 1,816 suspected cholera cases at the icddr,b Dhaka hospital and tested their stool samples using 7 diagnostic assays, including 5 commercially available cholera RDTs (Abbott, Cholkit, Artron, Crystal VC O1, Crystal VC O1/O139), PCR and culture, each on direct and APW-enriched specimens (e.g., ≥14 tests per patient). Overall, the sensitivity of direct RDTs ranged from 84.7%-97.6% and specificity ranged from 84.0%-98.7% across tests, nearing the estimated PCR sensitivity of 97.5% and specificity of 99.2% and exceeding culture sensitivity of 61.2%. Enrichment reduced RDT sensitivity (on average from 90.2% to 80.1%) and improved specificity (from 95.4% to 99.0%), with accuracy varying by age and antibiotic use. Older age groups exhibited higher RDT sensitivity than children <5 years across all tests with the largest differences observed for Abbott (direct: 89.6% vs. 96.6%). In addition, prior antibiotic use was associated with a substantial reduction in RDT sensitivity (direct: 91.9% without antibiotics vs. 85.7% with antibiotics; enriched: 82.6% vs. 73.9%). These findings carry significant implications for the use of RDTs in cholera surveillance and outbreak response and should be considered when interpreting results and selecting diagnostic testing strategies.

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GENOMIC EVOLUTION OF MULTIDRUG-RESISTANT (MDR) *VIBRIO CHOLERAE* FROM PERSISTENT OUTBREAKS IN KENYA

Samuel Kariuki[1], Diana Imoli[2], Cecilia Mbae[3], Wondwossen Gebreyes[4]

[1]DNDi and Kenya Medical Research Institute, Nairobi, Kenya, [2]Kenya Medical Research Institute, Nairobi, Kenya, [3]Kenya Medical Res Inst, Nairobi, Kenya, [4]Ohio State Univ College of Veterinary Preventive Medicine, Columbus, OH, United States

In October 2022, Kenya reported a resurgence of cholera with a case fatality rate (CFR) of 1.6%. We characterized 302 *Vibrio cholerae* 01 strains (2022-2025) from fecal samples of patients from an informal settlement, 20km East of Nairobi city. Susceptibility testing against 14 commonly available antibiotics was conducted using Kirby-Bauer disc diffusion method, extended spectrum beta lactamase production (ESBL) was confirmed using the Double Disc Synergy Test. Whole genome sequencing of 102 Multidrug-resistant (MDR) and ESBL positive was performed using Illumina NextSeq 2000 System. Genetic characterization was done using the Center for Genomic Epidemiology(CGE) and Pathogen watch platform, while AMRFinderPlus was used to identify resistance genes and mutations. All the outbreak isolates were identified as serotype Ogawa and were susceptible to meropenem, doxycline, amikacin, and chloramphenicol. Resistance to beta-lactam antibiotics was observed with 98% resistant to ceftazidime, 98.5% to cefotaxime, and 74.7% to cefepime. Additionally, 46.5% and 78.7% showed reduced susceptibility toamoxicillin/clavulanic acid and imipenem, respectively. Most of the isolates, 99% were MDR (resistant to at least one agent in three or more classes of the antibiotics tested) and 98.5% tested positive for ESBL production. All the 52 sequenced strains carried *bla*PER-7 ESBL and *var*G carbapenemase genes, had mutations in *gyrA* and *parC* genes and carried the *Inc*C plasmid. Genomic analysis of the sequenced strains indicated that they belonged to *V. cholerae* O1 El Tor Variant biotype, falling within late wave 3 of the ongoing seventh pandemic contained in the 13-transmission event (T13). These MDR strains persistent throughout the wet and dry seasons of cholera outbreak in our settings. There is need for continuous surveillance to monitor the evolution of MDR *V. cholerae* strains in endemic settings and consideration for deployment of therapeutic alternatives such as oral cholera vaccines. for prevention and control of outbreaks.

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RELIANCE ON SELF-REPORTED VACCINATION STATUS CAN BIAS OBSERVATIONAL STUDIES: INSIGHTS AND SOLUTIONS FROM CHOLERA STUDIES IN THE DEMOCRATIC REPUBLIC OF THE CONGO

Hannah Van Wyk[1], Patrick Musole Bugeme[1], Jules Jackson[2], Levi Bugwaja[1], Elizabeth Lee[1], Pengcheng Fang[3], Aybuke Koyuncu[3], Chloe Hutchins[4], Javier Perez-Saez[1], Jae-Woong Lee[5], Deok Ryun Kim[5], Esther German[4], Jackie Knee[4], Espoir Bwenge Malembaka[1], Andrew S. Azman[1]

[1]University of Geneva, Geneva, Switzerland, [2]Baltimore, MD, United States, [3]Johns Hopkins University, Baltimore, MD, United States, [4]London School of Hygiene and Tropical Medicine, London, United Kingdom, [5]International Vaccine Institute, Seoul, Republic of Korea

Accurate vaccination status classification is crucial for quantifying vaccination program quality through vaccine coverage (VC) and for estimating vaccine effectiveness (VE). Monitoring, evaluation, and research studies generally rely on self-reported vaccination status, which is known to be imperfect. Vaccine registries can provide additional, though still imperfect, information, but their comparative performance and how to best combine them with self-report data remain unclear. In this study, we estimated individual-level true vaccination status from case-control study

data of oral cholera VE in Uvira DRC by using a latent class model with self-report and vaccine registry data. We estimated the sensitivity and specificity of self-reported vaccination status and the vaccine registry and ran simulations to explore how these values bias VC and VE estimates. We then assessed how different strategies for combining self-report and registry data, specifically classifying disagreements between self-report and the registry as vaccinated or unvaccinated, influence bias in estimated VC and VE. We found that self-report had a higher sensitivity than the vaccine register (0.82 [95% CI: 0.76, 0.86] versus 0.73 [95% CI: 0.70, 0.77]), while the register was more specific (0.90 [95% CI: 0.86, 0.93] versus 0.70 [95% CI: 0.63, 0.76]). Relative to controls, self-report sensitivity was lower, but specificity was higher among cases. Using self-report alone produced VC and VE estimates up to 60 and 45 percentage points from the true values, respectively, with bias minimized at moderate VC (50-75%) and VE (30-40%). Cholera VE estimates that combine self-reported vaccination status and the registry would be, on average, around ten percentage points closer to true VE than estimates that use only self-report data. Our results highlight the magnitude of bias from relying on self-reported vaccination status and identify scenarios where a vaccine register would minimize bias. Given the logistical complexity and cost of vaccine registers, these findings inform their future implementation and use.

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EPIDEMIOLOGY OF *VIBRIO CHOLERAE* INFECTIONS IN THE HOUSEHOLDS OF CHOLERA PATIENTS IN THE DEMOCRATIC REPUBLIC OF THE CONGO: WASHMOBILE PICHA7 PROSPECTIVE COHORT STUDY

Presence Sanvura[1], Alves Namunesha[1], Jean-Claude Bisimwa[1], Kelly Endres[2], Willy Felicien[1], Camille Williams[2], Shubhanshi Trivedi[3], Kilee Davis[4], Jamie Perin[2], David Allen Sack[2], Justin Bengehya[1], Jean Claude Kulondwa[5], Ghislain Maheshe[1], Cirhuza Cikomola[1], Lucien Bisimwa[1], Daniel T. Leung[6], Alain Mwishingo[7], Christine Marie George[8]

[1]Université Catholique de Bukavu, Bukavu, Democratic Republic of the Congo, [2]Baltimore, MD, United States, [3]University of Utah - Division of Infectious Diseases, Lehi, UT, United States, [4]University of Utah, Salt Lake City, UT, United States, [5]Division Provinciale de la Santé Sud Kivu, Ministère de la Santé, Bukavu, Democratic Republic of the Congo, [6]Univ of Utah School of Medicine, Salt Lake City, UT, United States, [7]Bukavu, United States, [8]Johns Hopkins Bloomberg Sch of Pub Hlth, Baltimore, MD, United States

The cholera remains a major public health challenge in low- and middle-income countries. According to previous studies, household members of cholera patients are at high risk of developing cholera during the 7 days high risk period. The aim of this prospective cohort study is to build evidence on transmission dynamics and risk factors for *Vibrio cholera*e infections in cholera patient households in Democratic Republic of the Congo (DRC). During our cholera surveillance conducting at 115 healthcare facilities in South Kivu, we enrolled cholera patients and their household contacts, defined as individuals residing in the same home and sharing the same cooking pot with the cholera patient for the past 3 days. Household contacts of cholera patients were observed for 1-month after the index cholera patient was admitted to a healthcare facility for stool, serum, and water collection in urban Bukavu in South Kivu, DRC. A *V. cholerae* infection was defined as a *V. cholerae* bacterial culture positive result during the 1-month surveillance period and/or a four-fold rise in a *V. cholerae* O1 serological antibody from baseline to the 1-month follow-up. Twenty-seven percent of contacts (134 of 491) of cholera patients had a *V. cholerae* infection during the surveillance period. Significant risk factors for symptomatic *V. cholerae* infections among contacts were stored food left uncovered (Odds Ratio (OR): 2.39, 95% Confidence Interval (CI): 1.13, 5.05) (and younger age (children <5 years) (OR: 2.09, 95% CI: 1.12, 3.90), and a drinking water source with >1 colony forming unit *E.coli* / 100mL (OR:3.59, 95% CI: 1.46, 8.84) for V. cholerae infections. In conclusion,the findings indicate a high risk of cholera among contacts of cholera patients in this urban cholera endemic setting, and the need for targeted water treatment and hygiene interventions to prevent household transmission of *V. cholerae*.

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IMPORTATION-DRIVEN CHOLERA OUTBREAKS WITH EMERGING PLASMID-MEDIATED ANTIMICROBIAL RESISTANCE IN UGANDA: INSIGHTS FROM GENOMIC SURVEILLANCE

Ritah Namusoosa[1], Viola Kasone[2], Benedict Kanamwanji[1], William Senyonga[1], Aidarayaan Namukula[1], Lydia N. Bulyaba[1], Susan Nambozo[3], Miriam Nabukenya[1], Judith Ainemukama Kyokushaba[4], Grace Najjuka[1]

[1]Ministry of Health- Department of National Health Laboratory and Diagnostic Services, Kampala, Uganda, [2]Viola Kasone, kampala, Uganda, [3]National Health Laboratory and Diagnostic Services, Ministry of Health, Kampala, Uganda, [4]Kampala, Uganda

Cholera in sub-Saharan Africa is characterized by recurrent outbreaks, but the relative contribution of endemic transmission versus cross-border introductions remains unclear. In Uganda, genomic data are not routinely integrated into surveillance to clarify these patterns and guide control strategies. We hypothesized that country-level genomic analysis of *Vibrio cholerae* isolates would characterize transmission dynamics, lineage diversity, and antimicrobial resistance (AMR) with implications for elimination efforts. We conducted a retrospective review of cholera genomic data from the National Microbiology Reference Laboratory (NMRL), Uganda, analyzing whole genome sequences of isolates collected between 2018 and 2024. Uganda-specific genomic data from a published multi-country dataset were analyzed to generate country-level insights into transmission dynamics and policy implications. A total of 47 *V. cholerae* O1 genomes were included. All isolates belonged to the seventh pandemic El Tor lineage and clustered within the AFR13 lineage, indicating limited lineage diversity. Despite this, substantial phylogenetic diversity was observed, consistent with multiple independent introductions rather than sustained local transmission. Genomic profiles were largely conserved; however, isolates collected from 2020 to 2023 acquired additional AMR genes, including aad2, blaPER-7, mph(A), mph(E), mrs(E), and sul1, associated with an IncA/C multidrug resistance plasmid, suggesting horizontal gene transfer rather than lineage replacement. No genotypic resistance to tetracyclines was identified. These findings demonstrate that cholera in Uganda is driven by repeated introductions within a regional transmission network, with emerging plasmid-mediated AMR. Integrating genomic surveillance into routine systems can improve detection of transmission pathways and AMR emergence, inform targeted cross-border interventions, and accelerate progress toward cholera elimination in Africa by shifting control strategies from endemic-focused approaches to genomics-informed, cross-border interventions.

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MORBIDITY, MORTALITY AND LONG-TERM CONSEQUENCES ASSOCIATED WITH *CRYPTOSPORIDIUM* INFECTION AMONG CHILDREN WITH MODERATE-TO-SEVERE DIARRHEA (MSD) IN KENYA, 2008-2018

Richard Omore Onyando[1], Alex O. Awuor[1], Evans Apondi[1], Dilruba Nasrin[2], Jane Juma[1], John Benjamin Ochieng[1], Billy Ogwel[1], Karen L. Kotloff[2]

[1]Kenya Medical Research Institute, Kisumu, Kenya, [2]Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD, United States

Cryptosporidium is a key pathogen associated with moderate-to-severe diarrhea (MSD) and increased post-infection mortality in young children, particularly in Kenya. We estimated prevalence and assessed factors associated with *cryptosporidiosis* and mortality among children aged 0-59 months with MSD in rural western Kenya. We conducted a secondary analysis of two prospective, health center and community-based case-control studies: the Global Enteric Multicenter Study (2008-2012) and the Vaccine Impact on Diarrhea in Africa Study (2015-2018). MSD was defined as ≥3 loose stools in 24 hours plus ≥1 of the following: sunken eyes, skin tenting, dysentery, IV rehydration, or hospitalization within 7

days. Demographic and clinical data and stool samples were collected at enrollment and tested for enteropathogens using enzyme immunoassay. Deaths within ~60 days among children with *cryptosporidiosis* were assessed. Associations with mortality were evaluated using Chi-square/ Fisher's exact tests and Wilcoxon rank-sum tests. Among 3,332 children, 284 (8.5%) had *cryptosporidiosis*. The prevalence was higher in GEMS than in VIDA (195/1,778 [11.0%] vs 89/1,551 [5.7%], p<0.001) and declined with age (0-11 months: 158/1,415 [11.2%]; 12-23 months: 92/1,016 [9.1%]; 24-59 months: 34/898 [3.8%], p<0.001). Mortality among *Cryptosporidium* cases was 4.0% (95% CI: 2.0-7.1%). Children with *cryptosporidiosis* who died were younger (median age, months [IQR]: 10 [7-16] vs 14 [8-25], p<0.001) and had longer diarrhea duration (median days [IQR]: 7 [5-9.8] vs 6 [4-8], p<0.001). Death was also associated with higher likelihood of hospitalization (8/39 [20.5%] vs 3/234 [2.3%], p<0.001), moderate wasting (9/61 [14.8%] vs 2/212 [0.9%], p<0.001), and severe acute malnutrition (SAM) (6/26 [23.1%] vs 5/247 [2.0%], p<0.001). *Cryptosporidium* is an important driver of morbidity and mortality among children with MSD, particularly in malnourished infants in Kenya. Targeted child survival interventions for *cryptosporidium* should prioritize infants with MSD who present with malnutrition, wasting and longer diarrhea duration in this setting.

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SEASONAL RELATIONSHIP BETWEEN CLIMATE CONDITION AND INFANT ENTERIC INFECTIONS IN KENYA: INSIGHTS FROM SAFE START STUDY

Tahmidul Haque[1], Jane Awiti Odhiambo Mumma[2], Mark Janko[1], Kelly K. Baker[1]

[1]State University of New York at Buffalo, Buffalo, NY, United States, [2]Kisumu, Kenya

This study examined the association between climate conditions and infant enteric infections in Kisumu, Kenya, using data from the Safe Start cluster randomized trial of a food hygiene intervention. Stool samples (n = 1,493) were collected from infants at 22 and 39 weeks of age over a 15-month period (March 2018 to May 2019) to assess the prevalence of 18 enteric pathogens. Monthly prevalence was calculated as proportion of positive cases relative to the total monthly samples tested. Concurrent hourly weather data on temperature, dew temperature, precipitation, runoff and soil moisture were collected from ERA5 reanalysis satellite data for the 31 km (~0.25∘) grid including study communities and summarized as daily hourly averages. Highly skewed weather variables were log-transformed, and all variables were standardized. LOESS curves were drawn to explore non-linear temporal trends. All statistical procedures and visualizations were conducted using SAS 9.4. Weather variables demonstrated clear seasonal patterns, with peaks in moisture-related conditions occurring during the long and short rainy seasons (March-May and September-November), while temperature showed an inverse relationship with these parameters. Several pathogens, including Adenovirus, Campylobacter, Cryptosporidium, Norovirus, atypical enteropathogenic E. coli and selected bacterial and viral agents exhibited seasonal variation. McNemar's test demonstrated significant increase in pathogen prevalence between 22- and 39-weeks age, especially for Adenoviruses (18.43%), Campylobacter (17.63%) and atypical enteropathogenic E. coli (17.78%). Ongoing work includes conducting a factor analysis to identify discrete latent weather factors, followed by a distributed lag model to quantify the temporal association between climate conditions and infant enteric infections, adjusting for intervention effects. These findings highlight the role of seasonal environmental conditions in shaping enteric infection risk among infants and underscore the importance of timing targeted public health interventions to periods of heightened vulnerability.

6077

A PILOT PROSPECTIVE STUDY OF THE ESOPHAGEAL STRING TEST AS A MINIMALLY INVASIVE TOOL FOR THE DETECTION OF *HELICOBACTER PYLORI* AND ANTIMICROBIAL RESISTANCE GENES

Jessica Bass, Alla Turshudzhyan, Sanchita Das, Jia-Der Ju Wang, Michelle Makiya, Perla Adames Castillo, Michelle Luera, Sheila Kumar, Amy Klion

National Institutes of Health, Bethesda, MD, United States

Helicobacter pylori is a major global cause of chronic gastritis, peptic ulcer disease, MALT lymphoma, and non-cardia gastric cancer. Although prevalence has declined overall, infection remains common in many parts of Africa, Latin America, and East Asia where rates may exceed 70%. Eradication requires multidrug therapy, but rising antimicrobial resistance has increased treatment failure, now affecting up to 20% of cases. Resistance testing remains difficult because it typically requires invasive endoscopy and specialized culture methods with low recovery. We are conducting a prospective clinical study (NCT06871579) to evaluate the esophageal string test (EST) as a minimally invasive method for *H. pylori* detection and resistance assessment. Consented patients with active *H. pylori* undergo stool antigen testing and EST, with retrieval of the string after 1 hour. Quantitative PCR assays were developed to detect *H. pylori* and resistance-associated mutations in 23S rRNA and *gyrA*, associated with clarithromycin and levofloxacin resistance respectively, from the gastric portion of the string. Species-specificity was confirmed using cultured *H. pylori* and *H. bilis*. Patients are treated according to EST findings with test of cure 4 weeks after completion of therapy. To date, 14 of 20 planned patients have enrolled (median age 50.5 years, range 37-77; 50% female). 93% had epidemiologic exposure to high prevalence regions, and 11 had prior treatment failure, including 8 who had failed at least 3 regimens. EST has been completed in 10 patients; 9/10 (90%) were positive for *H. pylori*, including 2 patients with negative stool antigen testing but positive histology. Among positive samples, 23S rRNA mutations were detected in 7 of 9 and *gyrA* mutations in 7 of 9. Of 9 patients who have completed therapy, 8 achieved cure after first treatment course directed by trial results. EST was well tolerated, with only self-limited throat irritation reported. These preliminary findings support EST as a promising minimally invasive approach for *H. pylori* detection and antimicrobial resistance profiling.

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GUT MICROBIOTA-DRIVEN MODULATION OF IMMUNE RESPONSES AND PROTECTION IN INDIVIDUALS RECEIVING THE LIVE ATTENUATED ORAL ETEC VACCINE ACE527 AND SUBSEQUENT VIRULENT ETEC H10407 CHALLENGE

Soumya Basu[1], Ethan Gough[1], Jessica Brubaker[1], David Sack[1], August Louis Bourgeois[2], Richard Walker[3], Clayton D. Harro[1], Subhra Chakraborty[4]

[1]Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, United States, [2]PATH, Gaithersburg, MD, United States, [3]PATH, Washington, DC, United States, [4]Johns Hopkins University, Baltimore, MD, United States

Enterotoxigenic *Escherichia coli* (ETEC) is a major cause of diarrheal morbidity and mortality, particularly in low- and middle-income countries, with no licensed vaccines. Since majority of advanced ETEC vaccine candidates are oral ones, they must function within the complex gut microbiome. However, microbiome influence on vaccine-induced immunity remains poorly defined. We assessed microbiota-immune interactions in 27 healthy volunteers receiving two doses of the live attenuated oral ETEC vaccine ACE527 (21-day interval), followed by challenge with virulent ETEC H10407. ACE527 comprises three engineered strains expressing colonization factors and heat-labile toxin B (LTB), with demonstrated efficacy in challenge models. Faecal samples collected pre-vaccination (V0) and pre-challenge (C-1) underwent 16S rRNA sequencing. Microbial diversity (Shannon, inverse Simpson; Bray-Curtis, Jaccard) and composition

were analyzed alongside systemic and mucosal IgG/IgA responses to LTB and colonization factor antigen I (CFA/I). Associations were evaluated using regression, MiRKAT, and relaxed LASSO. The microbiota remained broadly stable, with some enrichment (~25-30%) of *Eubacterium_brachy_group*, *Family_XIII_AD3011*, and *Actinomyces*. Higher α-diversity (inverse Simpson) was inversely associated with mucosal IgA responses to LTB ($\beta = -0.72$; $p = 0.037$) and CFA/I ($\beta = -0.80$; $p = 0.023$). β-diversity metrics positively correlated with serum anti-CFA/I IgA fold changes ($p \approx 0.03$), suggesting compositional shifts enhance immune priming. Specific protective associations with reduced diarrhea and shedding were observed for members of *Peptostreptococcaceae, Oscillospiraceae, Lachnospiraceae, Veillonellaceae*, and Bacilli, whereas *Ruminococcaceae, Sutterellaceae, Coriobacteriia*, *Clostridia*, and *Actinobacteria* were linked to impaired responses. These findings highlight microbiota-mediated modulation of oral vaccine efficacy and inform strategies to improve immunization in high-burden settings.

6079

INDIGENOUS BELIEFS AND CHAGAS DISEASE IN COLOMBIA: RESURGENCE OF A DEADLY INFECTION

Lidia Gual Gonzalez

Clemson Unviersity, Clemson, SC, United States

Colombia has more than a hundred indigenous groups sparsely distributed across the country. Some of these communities live in areas where Chagas disease is endemic, causing a high disease burden among those populations. Due to anthropogenic activities and a lack of healthcare resources, the epidemiology of Chagas disease has shifted, and the effects are impacting indigenous communities at increased levels. The advancements in Colombian society have pressured the mitigation of Chagas disease, but the medical approaches rarely consider Indigenous beliefs and force upon them measures that do not align with their cultural traditions. This scenario has created tensions by undermining Indigenous knowledge and allowing a condescending treatment from the healthcare system, while at the same time failing to improve this public health problem. Although the inclinations from the healthcare system aim to help people and reduce disease burden, the lack of effective communication stalls their scientific progress. A One Health approach is needed to face this important problem, protecting humans, animals, and the environment, and designing sustainable solutions that prioritize the communities. Here we present the current situation in Colombia and discuss the existing cultural barriers that hinder medical advancements towards reducing Chagas disease, while also emphasizing Indigenous voices who have their own knowledge and beliefs around the interactions between the environment, diseases, and humans.

6080

APPLICATION OF CARDIAC MAGNETIC RESONANCE (CMR) IN EVALUATING CARDIAC INVOLVEMENT IN CHAGAS DISEASE: A SCOPING REVIEW

Carolina Guzman[1], Sylvia S. Sanchez[2], Shilah Faith Waters[3], Robert H. Gilman[4]

[1]Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, United States, [2]Baltimore, MD, United States, [3]Johns Hopkins University, Millcreek, UT, United States, [4]Johns Hopkins Univ Bloomberg School of Public Health, Baltimore, MD, United States

Chagas cardiomyopathy, a chronic manifestation of Chagas disease caused by infection with *Trypanosoma cruzi* infection, poses a major clinical burden due to high management costs and increased risk of long-termcardiac impairment. Early detection is essential, but tools to identify early myocardial damage are still needed. Cardiac magnetic resonance (CMR) is a non-invasive modality that provides detailed information on myocardial structure, function, and tissue characterization. This scoping review maps evidence on the use of CMR to assess cardiac involvement in Chagas disease. A comprehensive search was conducted in MEDLINE, EMBASE, Scopus, Web of Science, and Global Index Medicus. Rayyan was used for screening and Zotero for full-text review and data extraction. Three reviewers independently screened and extracted data. Studies up to October 2024 were included if they used CMR in patients with Chagas disease and had more than six participants. Non-human studies and those without full text were excluded. Thirty studies of a total of 1151 screenedwere included. Most participants were born in endemic regions of South and Central America, though not all resided there during the studies. Women were slightly more represented, and age ranged from 40 to 57 years. Cardiac involvement was often symptom-based, with palpitations most common, followed by chest pain, syncope, and dyspnea. Most patients were NYHA class I or II (asymptomatic or mild cardiac impairment). Most studies reported CMR imaging abnormalities. Late gadolinium enhancement, a marker of myocardial fibrosis commonly observed in patients with Chagas disease, ranged from 7% to 100% among patients mainly affecting the left ventricle, especially inferolateral and apical segments. Left ventricular dysfunction was more frequent. Other findings included apical aneurysms, wall motion abnormalities, myocardial edema, and increased extracellular volume. The scoping review demonstrates that CMR imaging provides a comprehensive evaluation of cardiac alterations in a single modality and can support early diagnosis of myocardial involvement.

6081

IMPROVING ACCESS TO DIAGNOSIS OF CUTANEOUS LEISHMANIASIS: DEVELOPMENT OF A RAPID MULTIPLEX RECOMBINASE POLYMERASE AMPLIFICATION-LATERAL FLOW TEST

Insaf Ben Hadj Ali[1], Yosser Zina Abdelkrim[1], Yusr Saadi[1], Ikram Rejeb[1], Nour Mohamed[1], Imen Khammari[2], Hejer Souguir[1], Emna Harigua-Souiai[1], Hamed Chouaieb[2], Meryem Lemrani[3], Aicha Kallel[4], Kalthoum Kallel[4], Nabil Haddad[5], Oussaima El Dbouni[6], Rhea N. Coler[7], Steven G. Reed[8], Akila Fathallah-Mili[2], Ikram Guizani[1]

[1]Laboratory of Molecular Epidemiology and Experimental Pathology-LR16IPT04, Institut Pasteur de Tunis, University of Tunis El Manar, Tunis, Tunisia, [2]Parasitology department, UH Farhat Hached, Faculty of Medicine of Sousse, University of Sousse, Sousse, Tunisia, [3]Institut Pasteur du Maroc, Casablanca, Morocco, [4]Parasitology department, UH La Rabta, Faculty of Medicine of Tunis, Tunis, Tunisia, [5]Faculty of Public Health, Lebanese University, Beirut, Lebanon, [6]Rafic Hariri Hospital, Beirut, Lebanon, [7]Seattle Children's Research Institute, Center for Global Infectious Disease Research, Seattle, WA; Department of Pediatrics, University of Washington School of Medicine, Seattle; Department of Global Health, University of Washington, Seattle, WA, United States, [8]HDT Bio, Seattle, WA, United States

Leishmaniases are neglected tropical diseases presenting in three main clinical forms: cutaneous, visceral, and mucocutaneous caused by different *Leishmania* species having similar morphology. Cutaneous leishmaniasis (CL) represents a significant public health concern worldwide including in Tunisia where it is highly endemic. Rapid parasite detection is essential for diagnosis, timely treatment and improved clinical outcomes, including reduced scarring and subsequent stigma. Furthermore, accurate species identification is critical for epidemiology, disease etiology and appropriate therapeutic management. Ensuring access to reliable diagnostic tools in endemic, resource-limited settings remains a major challenge. This study aims to deliver a rapid, accessible molecular diagnostic tool based on multiplex Recombinase Polymerase Amplification (RPA) coupled with lateral flow strip detection (LF). Screening assays were undertaken to select three species-specific primer pairs targeting *L. major*, *L. infantum*, and *L. tropica* that demonstrated high specificity in simplex RPA assays. Different primers combinations were tested in order to select the one(s) that enable species identification with high specificity. The best multiplex RPA fulfilling our criteria was selected and reaction conditions were optimized using a Taguchi experimental design to achieve maximal signal intensity with minimal background noise. The optimized assay enables clear discrimination among the three studied *Leishmania* species endemic in Tunisia. Amplification was achieved within 40 minutes, followed by detection within 10 minutes. The method showed good stability and reproducibility when evaluated on 28 well-documented *Leishmania* strains' DNAs from different species, geographical or host origins. The multiplex-RPA-LF is currently evaluated

on clinical samples to measure its performances (sensitivity, specificity, efficiency) for diagnosing CL with reference to direct examination and another classical molecular method.

6082

MIXED-EFFECTS MODELING REVEALS THE HETEROGENEOUS PROGRESSION OF CHAGAS CARDIOMYOPATHY IN AN ENDEMIC COHORT

Shilah F. Waters[1], Adriana Hernani-Rodrigo[1], Marcelo Andres Buhezo Chamon[2], Carolina Duque[1], Freddy Tinajeros[3], Sylvia S. Sanchez[1], David T. Martin[4], Hugo Buhezo[5], Rachel Marcus[6], Gustavo Duran[5], Robert H. Gilman[1]

[1]Johns Hopkins University, Baltimore, MD, United States, [2]Universidad Catolica Boliviana, Tarija, Plurinational State of Bolivia, [3]Asociacion Benefica PRISMA, Santa Cruz de la Sierra, Plurinational State of Bolivia, [4]Brigham and Womens Hospital, Boston, MA, United States, [5]Clinica de las Americas, Santa Cruz de la Sierra, Plurinational State of Bolivia, [6]LASOCHA (Latin American Society Of Chagas), Washington, DC, United States

Cardiomyopathy develops in approximately 40% of individuals with chronic *Trypanosoma cruzi* infection, and the ability to predict which patients will progress to severe disease is critical for prioritizing clinical resources. To identify prognostic markers of progression prior to disease development, we conducted a longitudinal study of adults recruited from a public hospital in Santa Cruz de la Sierra, Bolivia, between December 2014 and March 2020; participants were followed through April 2025. *T. cruzi* infection was confirmed using a combination of Indirect Hemagglutination Assay, ELISA, and TESA-blot testing. Participants underwent annual evaluations consisting of clinical interviews, electrocardiograms, and transthoracic echocardiograms. Severity was graded from Stage A (no observed abnormalities) to Stage D (structural cardiac disease with a left ventricular ejection fraction < 25%) using Brazilian Consensus criteria and 2022 AHA/ACC/HFSA guidelines. Missing information was accounted for using multiple imputation by chained equations. Mixed effects modeling with an unstructured covariance was applied to evaluate prognostic indicators of disease progression at the subject level. A total of 1,922 (1,598 seropositive and 324 seronegative) subjects were followed for up to 9 years. Accounting for clustering at the individual and family-levels, the strongest predictors of disease progression included age (OR = 1.60 per 5-year increase, $p<0.001$) and being male (OR = 2.72, $p<0.001$), consistent with previous publications. Importantly, there was substantial between-individual variability in baseline risk, exceeding clustering at the family level and variation in disease trajectories over time. This study reinforces that progression to determinate Chagas disease is highly heterogeneous across individuals, even after accounting for a robust set of covariates. This suggests that better clinical tools are needed to stratify individual patient risk of Chagasic cardiomyopathy progression.

6083

EVALUATION OF LAMP ASSAY COMBINED WITH PROTEINASE K DIGESTION FOR DETECTION OF *TRYPANOSOMA CRUZI* IN TRIATOMINE FECES

Luciana Lidia Basma Melgar[1], Franco Ignacio Soto Mercado[1], Dayana Nicole Serrano Olmos[1], Ana María Montaño Arias[2], Robert H. Gilman[3], Brandon N Mercado-Saavedra[1]

[1]Instituto de Investigación de Medicina, Universidad Católica Boliviana San Pablo, Santa Cruz de la Sierra, Plurinational State of Bolivia, [2]Unidad Funcional de Entomología Médica, Centro Nacional de Enfermedades Tropicales, Santa Cruz de la Sierra, Plurinational State of Bolivia, [3]Johns Hopkins Univ Bloomberg School of Public Health, Baltimore, MD, United States

Chagas disease is characterized by a chronic course with cardiac and gastrointestinal manifestations and it is a significant public health concern, particularly in rural areas of Latin America where it is endemic. It is caused by the parasite *Trypanosoma cruzi* which involves a complex cycle that includes mammals, humans, and vectors (triatomine). One of primary modes of transmission for this disease in endemic areas is vector-borne. Currently, direct microscopy of triatomine bug feces is commonly used in communities, although this method underestimates the positivity due to its low sensitivity. Molecular techniques, such as qPCR, have emerged as viable alternatives, offering sensitivity exceeding 90%; however, their high cost limits their accessibility. In this context, LAMP, an isothermal approach that does not need specialized equipment, is emerging as a more economical and simpler alternative to PCR, ideal for resource-limited settings. This study evaluates the diagnostic concordance of LAMP with other diagnostic techniques, aiming to establish it as an accessible and accurate option for diagnosing *T. cruzi* in. vectors. We used a *T. cruzi*-positive curve at a known concentration, extracted using a kit method and proteinase K digestion, and then diluted in a 1:10 to perform amplification via LAMP and conventional PCR. The results show that PCR could detect 10 parasites per milliliter of sample using the standard kit method. In parallel, LAMP could detect 100 parasites per milliliter of sample using the standard kit extraction method and less than 1 parasite per milliliter of sample using proteinase K digestion. Subsequently, a blinded study was conducted on 100 samples, of 32 samples tested positive by PCR. LAMP and PCR were applied in parallel to these samples, yielding 71.9% sensitivity and 100% specificity, comparable to PCR, with a kappa coefficient of 0.77. Furthermore, when compared with microscopy, LAMP combined with proteinase K digestion detected 28.6% more cases, establishing itself as a viable point-of-care option in areas with high transmission rates or as a simple alternative for entomological teams in hard-to-reach areas.

6084

PREDICTING RELAPSE IN VISCERAL LEISHMANIASIS: NOVEL INSIGHTS FROM AN INDIVIDUAL PATIENT DATA META-ANALYSIS

James Patrick Wilson[1], Prabin Dahal[1], Ahmed Mudawi Musa[2], Singh-Phulgenda Sauman[1], Rishikesh Kumar[3], Suman Rijal[4], François Chappuis[5], Fabiana Alves[6], Philippe Guérin[1], Krishna Pandey[3], Shyam Sundar[7]

[1]Infectious Diseases Data Observatory (University of Oxford), Oxford, United Kingdom, [2]Institute of Endemic Diseases, University of Khartoum, Khartoum, Sudan, [3]Rajendra Memorial Research Institute of Medical Sciences (Indian Council of Medical Research), Patna, India, [4]World Health Organization South-East Asia Region, New Delhi, India, [5]Faculty of Medicine, University of Geneva, Geneva, United Kingdom, [6]Drugs for Neglected Diseases initiative (DNDi), Geneva, Switzerland, [7]Institute of Medical Sciences, Banaras Hindu University, Varansai, India

Visceral leishmaniasis (VL) remains a highly neglected disease that disproportionately affects the poorest and most marginalised communities. Although treatment is often initially successful, 5–10% of patients relapse, posing a growing and under-recognised barrier to elimination. Despite this, no validated tools exist to stratify patients based on their relapse risk. Addressing this gap, we conducted the largest analysis to date of relapse predictors in the Indian subcontinent (ISC), leveraging data from 4,599 patients (228 relapses) enrolled in 19 prospective studies shared and harmonised by the Infectious Diseases Data Observatory (IDDO) platform. We developed two multivariable logistic regression models to predict relapse within six months of treatment: one including and one excluding pre-treatment parasite grade. Analyses were restricted to patients without VL/HIV co-infection. Candidate predictors, measured pre-treatment, included demographic, clinical, and laboratory variables. Study was modelled as a random effect; missing data were handled using multiple imputation; and internal validation was performed with bootstrapping. Predictors were selected using backward selection ($p < 0.10$). Across both models, relapse was associated with extremes of age, shorter fever duration, non-severe anaemia, and treatment with miltefosine (vs. single-dose liposomal amphotericin B). Pre-treatment parasite grade was a strong predictor when included. Optimism-adjusted c-statistics were 0.68 and 0.67 for models with and without parasite grade, with good calibration. This study represents the first large-scale, internally validated prediction models for VL relapse, enabled by a decade-long data-sharing collaboration between investigators and IDDO. These findings provide novel insight into

VL relapse biology and offer a practical framework for risk stratification, supporting targeted follow-up, improved trial design, and policy decisions within the ongoing ISC elimination programme.

6085

EVALUATION OF A FIELD-ADAPTABLE RPA-LF ASSAY FOR DETECTION OF *TRYPANOSOMA CRUZI* IN TRIATOMINE VECTORS IN BOLIVIA

Franco Ignacio Soto Mercado[1], Luciana Lidia Basma Melgar[1], Ana María Montaño Arias[2], Natalie M. Bowman[3], Brandon N Mercado-Saavedra[1]

[1]Instituto de Investigación de Medicina, Universidad Católica Boliviana San Pablo, Santa Cruz Campus, Santa Cruz de la Sierra, Plurinational State of Bolivia, [2]Centro Nacional de Enfermedades Tropicales (CENETROP), Santa Cruz de la Sierra, Plurinational State of Bolivia, [3]Univ of North Carolina, Chapel Hill, NC, United States

Chagas disease remains a major public health concern in Bolivia, where vector-borne transmission plays a central role in disease persistence. Effective detection of *Trypanosoma cruzi* in triatomine vectors is essential for surveillance and control; however, current diagnostic approaches, primarily based on optical microscopy of fecal samples, have limited sensitivity and may underestimate infection rates in endemic areas. This study aimed to standardize and evaluate a field-adaptable molecular diagnostic approach for the detection of *T. cruzi* in triatomine vectors, integrating simple DNA extraction methods with recombinase polymerase amplification coupled to lateral flow detection (RPA-LF). As part of this approach, three DNA extraction methods based on crude fecal lysates were assessed, with proteinase K digestion identified as the most reproducible approach. When combined with conventional PCR, this method achieved a limit of detection (LoD) of 100 parasites/mL, which was also observed when coupled with the RPA-LF assay. The RPA-LF method was preliminarily validated and compared to optical microscopy, using PCR as the reference standard. Using crude lysates, the assay showed a sensitivity of 71% and specificity of 89%, while purified DNA extracts improved performance to a sensitivity of 80% and specificity of 100%. In both cases, RPA-LF demonstrated higher sensitivity than microscopy and greater agreement with PCR. These findings support the potential of RPA-LF as a low-cost, field-adaptable molecular tool for vector surveillance, enabling improved detection of *T. cruzi* in resource-limited settings and strengthening early identification of infected vectors in endemic regions.

6086

SEGMENTAL MYOCARDIAL DYSFUNCTION AND OUTCOMES IN CHAGAS CARDIOMYOPATHY BEYOND EJECTION FRACTION: A CMR FEATURE TRACKING STUDY

Maria Daniela Valderrama Achury[1], Nicolás Ariza[2], Diego Rangel[2], Julian Francisco Forero[2], Carlos Eduardo Guerrero[2], Hector Medina[3], Matthias G. Friedrich[1], Michael Chetrit[1]

[1]RI - MUHC Research Institute, Montreal, QC, Canada, [2]Fundación Cardioinfantil- Instituto de Cardiología, Bogotá, Colombia, [3]Baylor Scott & White Health, Austin, TX, United States

Chronic Chagas cardiomyopathy (CCC) is characterized by myocardial dysfunction and ventricular aneurysms. Cardiac magnetic resonance feature tracking has demonstrated sensitivity for detecting early dysfunction in other conditions. However, the relationship between regional deformation patterns in CCC and clinical outcomes remains unclear. Retrospective analysis of patients with CCC referred for CMR evaluation(2016-2022). We developed the CHAMP strain ratio, defined as the average of apical and mid-ventricular segments divided by basal ones,to characterize regional deformation patterns.Outcomes were collected from medical records and telephone interviews. 55 patients were included (64 ± 11 years; 53% women).Higher CHAMP ratio values in patients without ventricular aneurysms and lower ratios were observed in those without, independent of left ventricular ejection fraction. During a median follow-up(2.6 years), 25 patients had the composite outcome (all-cause mortality, stroke, heart failure hospitalization, or cardiac transplantation). Patients with a low strain ratio (<1.58) showed a higher risk of the primary outcome with a HR of 2.46 (p=0.058). After adjustment for age, this association remained although not statistically significant (HR 2.16, p=0.118). Kaplan-Meier demonstrated earlier and more frequent events in patients with a low strain ratio compared with those with a high ratio (log-rank p=0.051). Device implantation was frequent in this cohort and a logistic regression analysis showed a low strain ratio associated with a significantly higher likelihood of device implantation (OR 3.68, p=0.038). Time-to-event analysis showed that patients with a low strain ratio had an increased risk of device implantation (HR 1.96, p=0.085),with a similar trend after adjustment for age. In conclusion,CHAMP ratio has the potential to identify segmental dysfunction in CCC and provide prognostic insights for adverse outcomes and device implantation. Despite the sample size, trends highlight the potential clinical utility of this index. Larger, multicenter studies are needed to validate.

6087

METABOLOMIC PROFILING OF VOLATILE ORGANIC COMPOUNDS IN *LEISHMANIA* SPECIES *IN VITRO*

Alex Benjamin Green[1], Anabel Zabala[2], Phillip Scott[2], Audrey R. Odom John[1]

[1]Children's Hospital of Philadelphia, Philadelphia, PA, United States, [2]University of Pennsylvania, School of Veterinary Medicine, Philadelphia, PA, United States

Approximately 1 billion people live in leishmaniasis-endemic regions. Cutaneous leishmaniasis causes 0.7-1.3 million cases annually, while visceral leishmaniasis affects 50,000-90,000 people annually and is 95% fatal without treatment. Pediatric cases comprise the majority cases. Contemporary diagnostics are invasive, costly, or insufficiently sensitive and specific. Volatile organic compounds (VOCs), chemoattractants for insect vectors, represent promising noninvasive biomarkers. We sought to characterize *in vitro* VOC profiles of *Leishmania major and L. infantum* to identify compounds with diagnostic potential. *L. major* and *L. infantum* promastigotes were cultured in Schneider's medium with 10% fetal bovine serum. Headspace VOCs were collected at 48 hours incubation via thermal desorption tubes and analyzed by two-dimensional gas chromatography and time-of-flight mass spectrometry. Peak alignment, normalization, and spectral matching to the NIST library were performed in ChromSpace software. Principal component analysis (PCA) was performed on scaled feature matrices. Group differences were assessed via permutational multivariate analysis of variance (PERMANOVA) and permutational analysis of multivariate dispersions (PERMDISP). A total of 21 VOCs were found differentially enriched between *Leishmania* cultures and media controls. PERMANOVA demonstrated significant multivariate differences ($R^2 = 0.94$, $p = 0.007$ for *L. major*; $R^2 = 0.98$, $p = 0.009$ for *L. infantum*). PERMDISP demonstrated small but significant within-group dispersion for *L. major*, and non-significant dispersion for *L. infantum*. *L. major* and *L. infantum* are associated with reproducible, statistically distinct VOC profiles under defined culture conditions. To our knowledge, this is the first characterization of *Leishmania*-associated VOCs *in vitro*. The pattern represents a fingerprint of parasite metabolic activity and highlights candidate biomarkers with diagnostic potential. These findings support further investigation of VOC-based tools for automated culture detection and noninvasive diagnosis.

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DECADAL EXPANSION OF *ANOPHELES* MOSQUITOES IN HIGH-ALTITUDE COMMUNITIES OF RURAL MADAGASCAR

Severine NS Raveloson[1], Rindra Rakotoarivony[2], Bernadette Rabaovola[3], Colman H. Shaver[4], Victor Razafindranaivo[2], Beauriche Andriambolaharijaona[2], Tolotra Ranarilalatiana[2], Edvin F. Randriambolamasina[5], Sarah Zohdy[6], Christina Bergey[7]

[1]Rutgers University, New Brunswick, NJ, United States, [2]University of Antananarivo, Antananarivo, Madagascar, [3]Centre ValBio Research Station, Ranomafana, Madagascar, [4]State University of New York at Stony

Brook, Stony Brook, NY, United States, [5]University of Antananarivo, Antananarivo, Madagascar, [6]Auburn University, Auburn, AL, United States, [7]Rutgers University, Piscataway, NJ, United States

Climate change is predicted to drive malaria-competent *Anopheles* mosquitoes to higher altitudes, placing previously low-risk highland communities at increasing risk of transmission. Madagascar's highland communities surrounding Ranomafana National Park span a steep elevation gradient (600-1200 m), making them a natural laboratory for detecting such shifts. Using collections from a 2013 baseline study as a reference, we investigated whether *Anopheles* abundance and community composition have changed across this gradient over the past decade. In 2023, we collected mosquitoes using CDC light traps at five villages (altitude 612-1133 m) in Ifanadiana District, Madagascar, sites overlapping with those surveyed in 2013. Mosquitoes were identified morphologically to genus. To characterize pathogen diversity and microbiome composition, we conducted metatranscriptomic sequencing of individual *Anopheles* specimens from each site for samples collected since 2023. The proportion of *Anopheles* among all mosquitoes collected increased dramatically between 2013 and 2023, from 27% (108/396) to 76% (643/848) across all sites. The most striking change occurred at Vohiparara, the highest-elevation site (1133 m), where *Anopheles* rose from 2.6% of collections in 2013 to 72.3% in 2023. Increases were observed across the elevation gradient, suggesting a broad community shift rather than a site-specific anomaly. We undertook metatranscriptomic analyses to characterize *Plasmodium* prevalence, co-infecting pathogens, and microbiome composition. Our data provide empirical evidence of rapid *Anopheles* range expansion into high-altitude communities in Madagascar over the past decade, consistent with climate-driven upslope mosquito migration. The change in *Anopheles* rarity at a site above 1100 m is of particular public health concern, as highland communities have historically lacked both malaria immunity and robust surveillance infrastructure.

6089

GENOME-WIDE SEQUENCE ANALYSIS OF MALARIA VECTORS REVEALS GENETICALLY ISOLATED *ANOPHELES COLUZZII* POPULATIONS ALONG THE SLOPES OF MOUNT CAMEROON

Kwi Pilate Nkineh[1], Brenas J. Jon[2], Apinjoh Obejum Tobias[3], Alfred Amambua Ngwa[1]

[1]MRC, Bakoa, Gambia, [2]WLiverpool School of Tropical Medicine, Liverpool, United Kingdom, Liverpool, United Kingdom, [3]University of Buea, BUEA, Cameroon

Anopheles gambiae complex is a major vector for the transmission of malaria parasites and other disease-causing pathogens. Unravelling the population structure of this vector could reveal crucial insights into parasite transmission dynamics and evolutionary adaptations that could inform strategies for disease control and elimination. This study explored whole genome sequence data of 276 *An. gambiae* complex mosquitoes collected between 2019 and 2020 in six communities across the altitudinal gradient of Mount Cameroon to determine the population structure of the complex. The different *An. gambiae* complex members were assigned into taxa based on known ancestral informative markers (AIMs). A set of 100,000 neutral biallelic single nucleotide polymorphisms (SNPs) were then randomly selected for the analysis of population structure and differentiation using Principal Component Analysis (PCA) and Fixation Index (Fst) respectively, followed by Admixture, Identity by Descent (IBD) and population dynamics analysis. Ninety-three (93), 178 and 4 of the vectors were identified as *An. coluzzii, An. gambiae s.s* and *An. melas* respectively while one was unassigned. Population structure revealed two subpopulations of *An. coluzzii* (referred to as *An. coluzzii* A and B), from the same locality (Missellele), indicating divergence despite probable sympatry, with the *An. coluzzii* B ancestrally closer to those from Burkina Faso. However, the *An. coluzzii* A sub-population was most differentiated from all other *An. coluzzii* populations, strongly driven by variants on the X and 2L chromosomes, suggesting local reproductive isolation and ecological adaptation. These genetic divergence and recent expansion of all species in all populations, call for further genomic surveillance to support locally tailored vector control and malaria elimination efforts

6090

EVIDENCE OF LIMITED MITOCHONDRIAL DIFFERENTIATION BUT PRONOUNCED NUCLEAR HAPLOTYPE STRUCTURING BETWEEN EASTERN AND WESTERN POPULATIONS OF *ANOPHELES FUNESTUS* IN TANZANIA

Emmanuel E. Hape[1], Letus L. Muyaga[1], Swalehe A. Masoud[1], Ismail H. Nambunga[1], Venter Nelius[2], Halfan S. Ngowo[1], Joel O. Odero[1], Lizette L. Koekemoer[2], Fredros O. Okumu[1]

[1]Ifakara Health Institute, Morogoro, United Republic of Tanzania, [2]Wits Research Institute for Malaria, Faculty of Health Sciences,University of the Witwatersrand, Johannesburg, South Africa

Anopheles funestus sensu stricto is one of the most important malaria vectors in sub-Saharan Africa. Its population genetic structure may be shaped by major ecological gradients and topographic barriers, with key implications for vector evolution and control. This study used mitochondrial and nuclear molecular markers to compare *An. funestus* populations from eastern Tanzania (coastal and southeastern zones) and western Tanzania (inland and Lake Zone), and to evaluate whether gene flow is restricted between these regions. Indoor-resting *An. funestus* were collected by CDC light traps from six sites: three in eastern Tanzania (Morogoro, Tanga, and Ruvuma; n=179) and three in western Tanzania (Mara, Shinyanga, and Katavi; n=166). Species identity was confirmed by PCR; only *An. funestus sensu stricto* were included. Mitochondrial clades (I and II) were identified using a *Taq*Man probe assay targeting mitochondrial SNPs. ITS2 D3 rDNA variation (~400 bp) was assessed by PCR amplification followed by *HpaII* restriction digestion; haplotypes (M, W, MW, Y, and Z) were assigned based on agarose gel banding patterns. Clade I predominated (70–87%); clade II was rare (0–26%) but higher in eastern sites (Ruvuma 26%, Morogoro 11%). In contrast, nuclear D3 haplotypes showed marked east-west differentiation. Eastern populations were dominated by Y-type (80% Morogoro) or M-type (64% Ruvuma), whereas western populations were characterized by MW (89% Shinyanga, 88% Katavi). These findings reveal limited mitochondrial but pronounced nuclear haplotype structuring, suggesting restricted gene flow. This pattern likely reflects topographic and ecological barriers, including the Great Rift Valley and climatic zonation. The observed population structure has important implications for region-specific malaria vector control, insecticide resistance management, and understanding the evolutionary dynamics of *An. funestus* in eastern Africa.

6091

CHROMOSOMAL INVERSIONS IN *ANOPHELES GAMBIAE* S.L. MOSQUITOES AND IMPLICATIONS ON MALARIA TRANSMISSION IN GHANA

Yaw Akuamoah-Boateng, Isaac K. Sraku, Judith D. Azumah, Grace A. Danquah, Anisa Abdulai, Abdul Rahim M. Sabtiu, Christopher M. Owusu-Asenso, Cornelia Appiah-Kwarteng, Fred Aboagye-Antwi, Patrick F. Ayeh-Kumi, Yaw A. Afrane

University of Ghana, Accra, Ghana

Anopheles gambiae sensu lato (s.l.) are rapidly adapting to heterogeneous environments through chromosomal inversions, which may enhance ecological plasticity, and sustain malaria transmission. However, little is known about current inversion distribution patterns and their epidemiological implications. This study investigated associations between inversion polymorphisms (2La, 2Rb), ecology of *An. gambiae* s.l. and their malaria transmission potential in Ghana. *Anopheles* immatures and resting adults were sampled in the Coastal savannah, Forest and Guinea-savannah ecological landscapes. Samples were speciated and karyotyped using PCR to determine inversion distributions in each landscape, association with vector resting behaviour, and *Plasmodium* infections. A high number of *An. coluzzii* sampled in the Guinea-savannah [2La (53.33%, 112/210),

2La/2L+a (30%, 63/210)] and Coastal [2La (30%, 60/200), 2La/2L+a (31%, 62/200)] landscape had inverted 2La karyotypes, probably to adapt and survive the warmer environmental conditions. The 2Rb inversion was observed in 76.67% (69/90) of *An. gambiae* s.s sampled in the Forest zone, reflecting adaptation to local environmental conditions. *An. gambiae* s.s. sampled in the highlands had either the inverted 2Rb (68%, 51/75) or heterozygote 2Rb/2Rb+ (32%, 24/75) karyotype, likely reflecting adaptation to desiccation stress associated with reduced humidity in highland environments. *An. coluzzii* that had the 2La inversion had lower odds of resting indoors, probably to avoid indoor insecticide pressure [OR=0.22, p<0.001]. *Plasmodium* infection was associated with the 2La inversion ($\chi^2(2)=34.50$, p<0.001), with the standard 2L+a karyotype higher in infected mosquitoes (50.57%, 44/87), whilst 65.52% (57/87) of the uninfected mosquitoes had inverted 2La karyotype. Study findings show a high number of *An. gambiae* s.l. in Ghana had inverted 2La and 2Rb chromosomes as adaptive response to unfavourable conditions, likely aiding their survival and disease transmission. Integrating inversion monitoring into routine vector surveillance could inform adaptive malaria control strategies.

6092

ALGAE-ASSOCIATED TRANSCRIPTOMIC RESPONSES IN WILD *ANOPHELES STEPHENSI* LARVAE FROM BREEDING SITES IN EASTERN ETHIOPIA

Ayomikun Emmanuel Aderounmu[1], Isuru Gunarathna[1], Babatunde Lawal Oriyomi[1], Katelyn McKindles[1], Solomon Yared[2], Gonzalo Vazquez-Prokopec[3], Tamar E. Carter[1]

[1]Baylor University, Waco, TX, United States, [2]Jigjiga, Ethiopia, [3]Emory University, Atlanta, GA, United States

The invasive malaria vector *Anopheles stephensi* has demonstrated remarkable ecological adaptability, facilitating its spread across diverse environments in the Horn of Africa. While environmental factors are known to influence mosquito development, abundance, and vector competence, the molecular mechanisms underlying these responses remain poorly understood. In particular, the role of aquatic microbial communities such as algae in shaping larval development and gene expression has received limited attention. In this study, we performed RNA sequencing (RNA-seq) on *An. stephensi* larvae collected from 12 natural breeding habitats across Jigjiga and Erer, Ethiopia, including 6 habitats with algal presence and 6 without. In addition, we identified several algal taxa present in these habitats, including dominant groups associated with each site. Principal component analysis (PCA) showed separation of samples by habitat type. Differential expression analysis identified 139 differentially expressed genes (DEGs) between algae-associated and non-algae habitats, with the majority (81%) upregulated in algae-associated samples. A subset of differentially expressed genes (n=3) was shared across both Jigjiga and Erer, while others were site-specific. Functional enrichment analysis identified pathways related to metabolism, redox processes, detoxification, and immune signaling. Enriched pathways included amino acid and carbohydrate metabolism, glutathione metabolism, and cytochrome P450-associated processes. These findings indicate that larval habitat characteristics, including algal presence, are associated with variation in gene expression in wild *An. stephensi*. These results provide a basis for understanding how environmental conditions in breeding sites may influence mosquito biology and could inform the development of more targeted vector control strategies. Further work is needed to determine the specific environmental drivers and functional consequences of these transcriptomic patterns

6093

GENOMIC SURVEILLANCE OF *AEDES AEGYPTI* CROSS EASTERN AFRICA REVEALS BACK-TO-AFRICA MIGRATION, REGIONAL GENETIC STRUCTURE, AND SPATIAL VARIATION IN INSECTICIDE RESISTANCE

Sandy Hickson

The University of Cambridge, Cambridge, United Kingdom

The global range expansion of *Aedes aegypti* out of Africa has placed more than half the world's population at risk of arbovirus infection. Yet this vector remains largely understudied across its native East African range, where arboviral outbreaks appear to be increasing in both frequency and intensity. Taxonomic evidence suggests this trend has coincided with the invasion of *Aedes aegypti aegypti (Aaa)*, the highly effective domestic vector of arboviral disease, into Eastern Africa, where it is suspected to be introgressing with the lower vectoral capacity, more sylvatic subspecies *Aedes aegypti formosus (Aaf)*. However, widespread admixture between these populations has not yet been genetically confirmed. We generated 383 whole-genome sequences sampled across 48 sites spanning Mozambique, Tanzania, and Kenya, and integrated these with 397 publicly available African *Ae. aegypti* whole-genome sequences, primarily from West Africa, for continent-wide population genomic analysis. We found evidence of *Aaa* introgression into Eastern Africa, with regional variation in ancestry components consistent with a recent back-migration of the domestic form. This back-migration is suspected to have introduced key anthropophilic traits associated with increased vectoral capacity, such as human-biting preference and domestic breeding-site selection. Genomic signatures of insecticide resistance were detected for all three major insecticide classes (organophosphates, carbamates, and pyrethroids) but occurred largely independently of regional genetic structure. We used identical-by-descent (IBD) segments to reveal fine-scale spatial patterns of gene flow within populations, estimating mean dispersal distance at 153-221 m - an estimate consistent with recent mark-release-recapture studies. This study underscores the importance of continued vector surveillance in East Africa, as the observed back-migration of anthropophilic *Aaa* may be increasing the vectoral capacity of *Ae. aegypti* populations across the region.

6094

UNDERSTANDING HERITABILITY IN THE RESPONSE TO DENGUE INFECTION BETWEEN AND WITHIN POPULATIONS OF *AEDES AEGYPTI*

Nina Dennington, Brianna McNulty, Suk Lan Ser, Matthew Jones, Tzu-Yi Lin, Elizabeth Ann McGraw

Pennsylvania State University, University Park, PA, United States

Aedes aegypti is the primary vector for dengue virus, which is responsible for the most common arboviral disease worldwide, dengue fever. With the continued rise in dengue fever, understanding the response to dengue infection is imperative to predicting disease dynamics. Here, we used a parent-offspring regression approach to quantify differences in heritability in viral load of dengue virus 1 and 2 within and between mosquito populations from Colombia and Brazil. Our results indicate relatively high broad-sense heritability in salivary glands (>72%) across populations and dengue serotypes. Notably, we show a significant narrow-sense heritability (96%) in Colombian mosquitoes infected with dengue virus 2. These data suggest variable heritability between mosquito populations and viral infection. To understand the molecular basis of these differences, we used RNAseq and whole genome analysis to compare the extreme phenotypic differences between high and low dengue families. Understanding the molecular basis which underpin heterogeneity in response to dengue infection would provide insights to future vector control strategies.

6095

TOWARDS AN UNDERSTANDING OF SPERM STORAGE IN FEMALE MOSQUITOES

Claudia A.S Wyer[1], Mehrnaz Afkhami[1], David Andrew Ellis[2], Sylvie Pitcher[1], Irene Alexandra Amaro[1], Patrick K. Perish[1], Jake W. Angelico[3], Emilie Mary Gray[4], Yasir H. Ahmed-Braimah[5], Mariana F. Wolfner[1], Laura C. Harrington[1]

[1]Cornell University, Ithaca, NY, United States, [2]University College London, London, United Kingdom, [3]Ithaca, NY, United States, [4]University of Notre Dame, Notre Dame, IN, United States, [5]Syracuse University, Syracuse, NY, United States

Aedes aegypti mosquitoes are important vectors of major arboviruses impacting human health including dengue, yellow fever, Zika and chikungunya. There is a desperate need for new control strategies for this mosquito including reproductive control. Females require only a single mating to store enough sperm for a lifetime of fertilization, capable of producing hundreds of viable- and potentially disease-transmitting-offspring. During copulation, males transfer sperm and seminal fluid proteins (SFPs) synthesized in the male accessory glands (MAGs) into the female bursa. Within minutes, sperm travel through the spermathecal ducts into the spermathecae, where they are stored and remain viable for the female's lifetime, likely sustained by secretions from spermathecal secretory cells (SSCs). However, the molecular mechanisms that enable SSCs to maintain sperm viability remain poorly understood. In this study, we used single-nucleus RNA sequencing (snRNA-seq) to profile individual cell types within the spermathecae, confirming SSC populations through RNA fluorescence *in situ* hybridization (FISH). We then examined the transcriptional response of SSCs to natural mating and MAG lysate injection, using virgin and saline-injected females as controls. Our results indicate that natural mating triggers a stronger transcriptional response in SSCs compared to MAG injection alone, suggesting that sperm receipt is a key factor in SSC activation. These findings provide new insights into the cellular and molecular dynamics of sperm storage in *Aedes aegypti*.

6096

ENGINEERING TRANSGENIC MOSQUITOES WITH *PLASMODIUM FALCIPARUM*-INDUCIBLE MORTALITY FOR TARGETED MALARIA CONTROL

Houhong Zhang, Yuemei Dong, Shengzhang Dong, Emelia Pearson, Sida Li, George Dimopoulos

Johns Hopkins University, Baltimore, MD, United States

Malaria remains a critical global public health challenge. With increasing insecticide resistance in mosquito vectors and drug resistance in Plasmodium parasites, novel vector control strategies are urgently needed. We are exploring the development of a novel transgenic mosquito-based malaria control concept using a *Plasmodium* infection-inducible mosquito mortality system that selectively kills infected mosquitoes or suppresses their vectorial capacity. Our approach uses a binary transgenic system in Anopheles mosquitoes. One component expresses an inactive protoxin composed of two insecticidal toxins linked by an NS2B/NS3-cleavable motif under a blood meal-inducible promoter. The second component expresses the NS2B/NS3 protease under a Plasmodium infection-inducible promoter. In infected mosquitoes, protease-mediated cleavage activates the protoxin and triggers targeted mosquito mortality. We identified several arthropod-derived, insect-specific toxins with strong insecticidal activity and stability that are capable of effectively killing mosquitoes. Four transgenic mosquito lines have been successfully established, each carrying a construct in which the protoxin is driven by a blood meal-inducible promoter and the activator protease is driven by a *Plasmodium* infection-inducible promoter, providing a foundation for subsequent functional evaluation of the system. Our approach offers a highly selective and robust genetic approach to reduce malaria transmission by specifically eliminating infected vectors or reducing vectorial capacities, thus potentially leading to a novel malaria control strategy.

6097

OPERATIONALIZING EQUITY IN AI FOR LOW-AND MIDDLE-INCOME COUNTRY (LMIC) HEALTH SYSTEMS: A FULL-STACK FRAMEWORK AND EVIDENCE SYNTHESIS

Rahima F. Dosani

Kinaura Partners, New York, NY, United States

Artificial intelligence (AI) is increasingly deployed across low-and middle-income country (LMIC) health systems, including clinical decision support, surveillance, and supply chain optimization. However, many systems are developed using data and design assumptions that do not reflect LMIC contexts, resulting in systematic bias across populations, geographies, and use cases which undermines accuracy, trust, and impact. We developed a six-layer "Equity Stack" framework to operationalize equity in AI for health: data, model development, applications and use cases, governance and policy, funding and infrastructure, and people and institutions. We conducted a structured synthesis of 18 peer-reviewed studies to identify five primary domains of bias: demographic, socioeconomic, geographic, linguistic and cultural, and digital access. These domains were mapped across the six layers to identify points of failure and intervention. Findings were triangulated with expert interviews and case studies from African health systems, including LLM adaptation for maternal health and development of open-source African language datasets. Bias was found to propagate across all layers, with compounding effects when unaddressed early in system design. Key failure points included non-representative training data, reliance on external benchmarks, and limited local governance and technical capacity. Case evidence shows that locally grounded model development and language adaptation improve both performance and user trust, particularly among underserved populations. We further estimate that one-third of potential AI-driven health system value in LMIC contexts is contingent on addressing these sources of bias, based on modeled value pools and bias-adjustment factors derived from the literature synthesis. The Equity Stack framework enables targeted interventions at each layer. The framework introduced helps shift equity from a downstream safeguard to an upstream design requirement. Embedding equity across the full stack is essential to realizing AI's benefits for LMICs while avoiding amplifying existing disparities.

6098

OPERATIONALIZING LOCALIZATION THROUGH CAPACITY STRENGTHENING IN GLOBAL FUND MALARIA GRANTS: A CASE STUDY OF GUINEA'S NATIONAL MALARIA CONTROL PROGRAM

Tamba Emile Sandouno[1], Felicien Randriamanantenasoa[1], Alioune Camara[2], Kabinè Diané[2], Ibrahima Sory Condé[2], Binnè Camara[2], Njara Rakotonirina[3], Monika Lussier[3], Lokesh Banydeen[3], Ana Brou Santiago[3], Donatien Ntambue[3], Chrestien Yameni[4]

[1]Catholic Relief Services, Conakry, Guinea, [2]National Malaria Control Program, Conakry, Guinea, [3]Catholic Relief Services, Baltimore, MD, United States, [4]Catholic Relief Services, Dakar, Senegal

In many countries in sub-Saharan Africa, Donor compliance often centralizes decision-making in international organizations, limiting national ownership. To boost local ownership and self-reliance in implementing global fund and other donors funded project in Guinea, CRS launched its High-Performing Implementers (HPI) in collaboration with the Guinea Ministry of health in 2021. The HPI initiative aims at using annual self-assessments aligned with Global Fund capacity standards across governance, finance, monitoring, evaluation, and supply chain management from 2021 to 2025. Support combined three components: capacity strengthening, development of tools, procedures, and systems, and continuous on-the-job coaching linked to annual action plans. This longitudinal case study assesses whether Catholic Relief Services' High-Performing Implementers (HPI), implemented with Guinea's National Malaria Control Program, operationalizes localization within Global Fund malaria grants by strengthening governance and expanding budgetary autonomy. Results show sustained improvements. Governance increased

from 60% to 86%, finance management from 47% to 73%, monitoring and evaluation from 59% to 68%, and supply chain from 48% to 70%. In parallel, grant budgets directly managed by the national program rose from USD 880,451 in 2022, largely staff salaries, to USD 4,256,233 in 2025 covering core functions. These shifts demonstrate transfers of control over financial management, monitoring, and supply chains. Findings suggest that nationally led self-assessment, paired with sustained system, skill, and coaching investments, can translate localization into operational and financial autonomy for malaria grant transitions.

6099

SUSTAINABLE THERAPEUTIC FEEDING PRACTICES TO ENHANCING CHILD NUTRITION AN EXPLORATORY STUDY IN WEST MAMPRUSI MUNICIPALITY, GHANA.

Doreen Kada Abagali
Braveaurora, walewale, Ghana

Child malnutrition is a critical global issue, contributing to nearly half of all deaths among children under five. In Ghana, stunting and underweight conditions remain prevalent, necessitating targeted interventions to address the Sustainable Development Goal (SDG) 2.2. Management of Severe Acute Malnutrition (SAM) in children in Ghana, largely depends on donor supported Read to Use Therapeutic Feeds (RUTF) provides by organizations like UNICEF. However, Whenever the health service stops receiving Read to Use Therapeutic Feeds (RUTF) from donors, they often rely only on nutrition counselling to manage SAM in children. This exploratory study assessed a community-based intervention combining health worker training, caregiver education, and household food production to promote sustainable child nutrition practices.The intervention is implemented in 13 communities,3 healthcare facilities in the west Mamprusi Municipality. A participatory workshop trained 12 healthcare workers on preparation and use of locally made therapeutic feed using ingredients like groundnut paste, vegetable oil, powdered milk and mineral mix to manage SAM.90 caregivers participated in food demonstration focused on preparing nutrient dense complementary foods using locally available ingredients. 90 Caregivers were supported to establish backyard gardens to improve access to variety of nutritious food crops. The findings showed improved knowledge and capacity among healthcare personnel to manage SAM using local available therapeutic feeds, alongside increased caregiver skills in preparing nutrient dense meals for children. The intervention also strengthened house hold awareness on nutrition sensitive food production through the backyard gardening. These results offer actionable insights for tackling child malnutrition through community-driven and sustainable solutions, providing a framework for scaling similar interventions across regions to reduce dependency on donor support for child nutrition, build local capacity, and improve child nutrition.

6100

PREVALENCE AND THE BEHAVIOURAL AND ENVIRONMENTAL DETERMINANTS OF ANAEMIA AMONG ADOLESCENTS IN RURAL AGINCOURT, NORTHEAST SOUTH AFRICA: A CROSS-SECTIONAL STUDY

Eva D. Mathatha, Kathleen Kahn, Shane A. Norris, Nicola Christofides, Chodziwadziwa Kabudula
University of the Witwatersrand, Johannesburg, South Africa

Anaemia remains a public health concern among adolescents in low- and middle-income countries, yet evidence on sex-specific determinants in rural South African settings is limited. Understanding social, dietary, and biological factors associated with anaemia during adolescence is essential for informing effective interventions. This study assessed the prevalence and environmental determinants of anaemia among adolescents in a rural South African setting. We analysed cross-sectional data from 461 adolescents aged 14-19 years (230 males and 231 females) in rural Agincourt, northeast South Africa. Anaemia was defined using World Health Organization haemoglobin cut-offs. Socio-demographic, household, dietary, environmental, and menstrual-related factors were assessed. Crude odds ratios (COR) were estimated using bivariate logistic regression, and multivariable logistic regression was used to estimate adjusted odds ratios (AOR) for combined-sex and female-only models. Overall anaemia prevalence was 27%, with no difference between males and females. In the combined model, late adolescence was associated with lower odds of anaemia (AOR = 0.57; 95% CI: 0.37-0.89), as was purchasing household food rather than relying on subsistence production (AOR = 0.32; 95% CI: 0.15-0.68). In the female-only model, menstruation lasting more than three days was associated with higher odds of anaemia (AOR = 3.35; 95% CI: 1.44-7.76), while frequent consumption of animal-source foods and purchasing household food were protective. Anaemia among adolescents in rural South Africa is common and shaped by age, household food sources, dietary practices, and menstrual health. Integrated adolescent-focused strategies addressing nutrition and routine anaemia screening are urgently needed.

6101

POSTPRANDIAL GLYCEMIC RESPONSES TO TRADITIONAL GHANAIAN MIXED MEALS

Emmanuella Awedana Apuri
Ghana Infectious Disease Centre, Accra, Ghana

The glycemic index (GI) is commonly used to classify carbohydrate-containing foods based on their impact on blood glucose levels after consumption. This guide provides dietary recommendations for managing metabolic disease. However, its applicability to real-world diets, particularly mixed meals commonly consumed in Sub-Saharan Africa including Ghana remains uncertain. In Ghana, staples such as ampesi, fufu, banku, and Ga kenkey constitute the daily diet for millions. Yet potentially erroneous GI-based assumptions may lead to suboptimal clinical guidance for an increasingly diabetic population. We conducted a controlled, repeated-measures crossover study among 10 non-diabetic adult participants in Ghana. Following a standardized overnight fast, participants consumed each of four traditional mixed meals on separate days in randomized order. Capillary blood glucose was measured at baseline and at 30-minute intervals over a 3-hour postprandial window. The results showed that the mean total peak area under the glucose response curve for Banku was the highest, while Ampesi had the lowest. Fermented preparations (banku, Ga kenkey) exhibited distinct late-phase glucose retention profiles, suggesting that food processing modulates postprandial kinetics beyond what GI values predict. The findings from this study suggest that glycemic response patterns, rather than the glycemic index alone are more useful for personalized diet and insulin planning and should be adapted to culturally specific diets.

6102

UNPACKING SDG INDICATOR 16.2.1 ON CHILD VIOLENCE EXPOSURE IN THE HOME: PREDICTORS OF “BEATING” AS A CHILD DISCIPLINE IN AFGHANISTAN

John D. McLennan
University of Calgary, Calgary, AB, Canada

The prevalence of children exposed to “violent discipline in the home” is indexed by SDG Indicator 16.2.1. This indicator captures any of eight different types of discipline that range in severity, with one clearly the most severe, i.e., whether a child has been “beat up, that is, hit over and over as hard as one could”. It is hypothesized that the predictors of “beating” may differ from other types of punishments counted in this SDG indicator. Determining this is particularly relevant if intervention initiatives include a harm reduction component. The aim of this study was to determine predictors of beating compared to other physical punishments of children in Afghanistan, a country with a high prevalence of use of “any” physical punishment. Data from the nationally representative 2022/3 Multiple Indicator Cluster Survey for Afghanistan were used, specifically adult caregiver responses to questions on the exposure of one randomly selected child in the home between 5-14 years of age to different types of physical punishment. “Beating” prevalence was 18.7% based on a sample of 15,891

households. In an adjusted logistic regression model, variables significantly associated with higher odds ratios of having been "beat" among those who had at least some exposure to physical punishment were (a) child being male, (b) caregiver belief in the necessity of physical punishment, (c) lower household wealth, and (d) more children in the home. In the same analysis, (e) younger child age, (f) biological mother in the home, (g) younger caregiver age, and (h) greater crowding in the home were not associated with being "beat", although these same variables (i.e., e-h) were associated with exposure to "any" physical punishment compared to none. Although caution is required in interpreting the predictor findings in this study, especially given the cross-sectional nature of the data, some patterns may inform next steps. A more comprehensive examination of caregiver beliefs about physical punishment, and "beating", might be prioritized given that this variable had the highest odds ratios for predicting both "any" physical punishment, and being "beat".

6103

LEADING FOR IMPACT: STRENGTHENING LEADERSHIP FOR CHILD HEALTH PROGRAM IMPLEMENTATION IN LOW-RESOURCE SETTINGS

Stephanie L. Enkel[1], Asha C. Bowen[1], Mickey Chopra[2], Keith Cloete[3], Alyssa B. Sharkey[4]

[1]The Kids Research Institute Australia, Nedlands, Australia, [2]The World Bank, Washington DC, DC, United States, [3]Department of Health, Western Cape Government, Cape Town, South Africa, [4]Princeton University, Princeton, NJ, United States

Many communicable disease programmes are technically sound yet fail to achieve sustained reach and impact in low-resource settings. We hypothesise that this reflects implementation challenges shaped by leadership, trust, local decision-making and broader health system constraints that are rarely examined directly. Led through a collaboration between The Kids Research Institute Australia, the University of Western Australia, Princeton University, the World Bank and health system partners, this study will investigate how leadership and community engagement influence delivery of communicable disease-focused child health programmes in systems under pressure, with a focus on prevention-oriented interventions targeting high-burden infectious diseases. A desk-based review will synthesise implementation science, leadership development and health systems literature to identify how leadership is conceptualised, supported and measured in low-resource settings. Approximately 30 key informant interviews will then be undertaken with programme implementers, policymakers, community members and leadership programme alumni across selected case sites, including settings transitioning from donor dependency. Data will be analysed thematically to examine how formal governance, adaptive leadership, relational trust and community-led problem-solving shape programme delivery. The study is designed to move beyond narrow assessments of programme fidelity to better understand the values, relationships and enabling conditions that support effective and equitable implementation. Findings will inform a practical, values-based leadership framework and co-designed tools for implementers and policymakers seeking to strengthen communicable disease prevention and child health delivery in low-resource settings. By the time of presentation, this work will have produced a draft framework to guide interview design and case selection. ASTMH will provide an important forum to refine the framework with global health practitioners and researchers, and to strengthen networks for the interview phase of the study.

6104

IMPACT OF SOCIAL MEDIA USE ON MENTAL HEALTH, CYBERBULLYING AND ACADEMIC ATTAINMENT AMONG YOUTH: A SCOPING REVIEW

Nazish Masud, Naomi Reyes, Patra C. Ezeamii, Tilicia L. Mayo-Gamble, **Isaac Chun Hai Fung**

Georgia Southern University, Statesboro, GA, United States

Social media use (SMU) among youth warrants research about its impact on their well-being, safety, and academic success. This scoping review aims to summarize SMU's impact on mental health, cyberbullying, and academic achievement in children aged 8 to 17 years old. All relevant literature published between Jan 1, 2021, and Sep 15, 2025, across 4 databases: PubMed, PsycINFO, ERIC, and Scopus, was identified. An online review management platform, 'Rayyan', was used to conduct a blind review by 3 independent reviewers. Ninety studies were included after full-text review. Among these, 55 focused on mental health, 13 examined academic attainment, and 22 addressed the issue of cyberbullying. There is emerging evidence linking problematic/addictive SMU, passive consumption, and high duration (> 4-6 h/day) to increased depressive symptoms, anxiety, poor sleep, and suicidality among youth. Excessive online sharing and emotional investment were common among girls, making them vulnerable to cyber victimization. The fear of missing out was a common reason for compulsive SMU among both genders. The more visual and photo-dependent platforms increased anxiety and depression due to low self-esteem and dissatisfaction. Boys were common perpetrators and reported involvement in cyberbullying. Parental monitoring and supervision are significant protective factors that help prevent the negative impacts of SMU. In-class checking and passive consumption of SMU are negatively associated with academic success and lower grades. This relationship is frequently mediated by impaired attention control, academic procrastination, and sleep deprivation due to increased online presence. This scoping review concludes that problematic SMU, passive consumption, high duration, and excessive sharing consistently predict poorer mental health, increased cyberbullying involvement, and reduced academic performance in youth. Policies aimed to prevent or reduce the negative implications of SMU should focus on integrating digital literacy programs in schools. Informed decisions and healthy media choices can prevent major problems arising from excessive SMU.

6105

ASSOCIATION BETWEEN HOUSEHOLD FLOORING MATERIAL AND PERINATAL MORTALITY IN LOW- AND MIDDLE-INCOME COUNTRIES

Gabby Barratt Heitmann[1], Anna T. Nguyen[1], Britney Bennett[1], ABUL KASHAM MD. SHOAB[2], Md. Rezaul Hasan[3], Ayse Ercumen Ercumen[4], Md Mahbubur Rahman[3], Gary Darmstadt[1], Farjana Jahan[5], Jade Benjamin-Chung[1]

[1]Stanford University, Stanford, CA, United States, [2]DHAKA, 13, Bangladesh, [3]ICDDR,B, Dhaka, Bangladesh, [4]North Carolina State Univ, Raleigh, NC, United States, [5]International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Dhaka, Bangladesh

Unimproved dirt floors remain common in low- and middle-income countries (LMICs). They may harbor pathogens that elevate infection risk during pregnancy, home delivery, and the postnatal period, making household flooring a plausible yet understudied modifiable risk factor for perinatal mortality. Approximately 28% of births in LMICs occur at home, where floor material may directly influence perinatal infection risk. We pooled DHS birth history data for singleton pregnancies from 2005-2023 from 69 surveys in 39 LMICs (N=489,253). We defined finished floors according to DHS guidelines (rudimentary and natural floors were unfinished). We estimated 1) the average treatment effect (ATE), i.e. the difference in perinatal mortality rates if all households had finished floors vs. none, and 2) the population attributable fraction (PAF), i.e., the % of perinatal deaths that would be prevented if all household floors were finished. We used inverse probability weighting to account for confounding by household

socioeconomic status, maternal age, education, and household size and obtained confidence intervals by bootstrapping (N=1,000 replicates). Estimates were weighted by DHS sampling weights and scaled by each country's population of women aged 15-49 years. The weighted prevalence of finished floors was 49.1% (range 19.2%-89.5% across surveys). Weighted mortality rates per 1,000 pregnancies were 14.6 for stillbirth, 23.9 for neonatal, and 38.5 for perinatal deaths. Finished floors were associated with lower stillbirth (ATE: -2.87 per 1,000 pregnancies, 95% CI: -5.25, -0.43), neonatal mortality (ATE: -3.85 per 1,000, 95% CI -7.09, -0.11), and perinatal mortality (ATE: -6.54 per 1,000, 95% CI: -10.75, -2.02). An estimated 10.0% (95% CI: 1.5%, 18.3%) of stillbirths, 7.6% (95% CI: -2.6%, 14.9%) of neonatal deaths, and 8.5% (95% CI: 2.7%, 14.0%) of perinatal deaths were attributable to unfinished floors. Residual confounding is possible, as finished floors are a marker of socioeconomic status. Household flooring upgrades are durable environmental intervention that may meaningfully reduce perinatal mortality in LMICs.

6106

THE EFFECT OF CO-IMPLEMENTATION OF SEASONAL MALARIA CHEMOPREVENTION WITH VITAMIN A SUPPLEMENTATION ON OTHER MATERNAL, NEONATAL AND CHILD HEALTH WEEK SERVICES IN NIGERIA

Olusola B. Oresanya[1], Ebenezer C. Ikechukwu[1], Ferdinand Ekeh[2], Umar Suleiman[2], Olabisi Adebo Ogunmola[3], Jennifer Ugochi Chukwumerije[4], Chibuzo Oguoma[2], Kolawole Maxwell[2], Chuks Nnaji[1], Christian Rassi[1]

[1]Malaria Consortium, London, United Kingdom, [2]Malaria Consortium, Abuja, Nigeria, [3]Malaria Consortium, FCT, Nigeria, [4]Malaria Consortium, Abuja, FC, Nigeria

Use of the well-established Seasonal Malaria Chemoprevention platform for additional child survival interventions is gaining interest. In Nigeria, vitamin A supplementation (VAS) is primarily delivered through biannual Maternal, Newborn and Child Health Weeks (MNCHWs). VAS coverage declined from 45% in 2018 to 37% in 2025 with a persistent urban-rural coverage gap. Co-delivering SMC and VAS could improve reach, however, there were concerns that this might reduce uptake of other services offered through MNCHWs. We conducted a one-arm multiple pretest/posttest mixed-methods evaluation of a co-delivery project in two Nigerian states, in which one round of VAS was delivered through SMC and another through the MNCHW. We tested the null hypothesis that uncoupling VAS from MNCHW does not affect coverage of other MNCHW delivered services. In Bauchi, household survey results showed MNCHW rounds without VAS had significantly lower coverage for deworming (16.8% vs. 20.0%, $p < 0.0001$), mid-upper arm circumference (MUAC) screening (14.0% vs. 21.3%, $p < 0.0001$), intermittent preventive treatment in pregnancy (IPTp) (28.4% vs. 32.1%, $p = 0.048$), tetanus toxoid (17.6% vs. 29.7%, $p < 0.0001$), and most vaccine antigens, except OPV1&2 and Measles 1, which did not change. Coverage of iron–folate supplementation (34.3% vs. 31.7%, $p = 0.584$), and other vaccines were also unchanged. In Niger, MUAC (41.6% vs. 41.0%, $p = 0.788$), IPTp (61.3% vs. 63.2%, $p = 0.508$), and tetanus toxoid (29.6% vs. 27.2%, $p = 0.678$) showed no significant differences, but deworming declined (47.1% vs 49.5%, $p = 0.001$), iron-folate improved (63.5% vs. 21.3%, $p < 0.0001$), and all vaccine antigens recorded higher coverage when VAS was co-delivered with SMC. Notably, VAS coverage itself increased significantly from 75.0% to 83.3% in Bauchi and 80.4% to 89.2% in Niger. These findings suggest that uncoupling VAS from MNCHW, while improving VAS coverage, may have variable effects on other MNCHW services. However, the magnitude and direction of effects could be context-dependent, warranting careful planning and monitoring in future SMC-VAS integration and MNCHW efforts.

6107

PROFILE OF NEONATAL MORTALITY IN A TERTIARY CARE CENTRE - GHANA, 2024

Akua Boadiwaa Amoh-Yeboah, Dora Dadzie, Donne Kofi Ameme

Ghana Field Epidemiology and Laboratory Training Program, Accra, Ghana

Despite low-cost interventions, neonatal mortality remains high in developing countries, where data needed to guide site-specific interventions are also lacking. We described the causes and characteristics of neonatal mortality in a tertiary care facility in Ghana to guide interventions. We abstracted data on neonatal mortalities from the special care baby unit for the period 2024. Neonatal death in Ghana is defined as death within the first 28 days of life, of an infant delivered after 28 weeks gestation. We calculated facility neonatal mortality rate (NMR), defined by the number of neonatal deaths per 1000 live births. Overall, 1184 neonates were hospitalized, out of which 177 (15%) died. Of mortalities, 60% (100/166) were males and 45% (75/168) were preterm. The facility NMR was 56 per 1000 live births (177/3138). Prematurity (324/1184, 27%), asphyxia (247/1184, 21%) and infectious diseases (260/1184, 22%) were the commonest indications for admission, with case fatality rates of 13% (41/324), 18% (44/247) and 13% (34/260) respectively. About 23% (38/168) of mortalities occurred on the first day of life, and 52% (87/168) within 24 hours of admission. The fatality was high for neonates referred (99/332, 30%). Majority (103/156, 66%) of neonates dying had hypothermia at presentation. Prematurity, asphyxia and infections were the commonest reasons for neonatal admissions and deaths in the tertiary care facility. Health workers were re-trained on essential obstetric and newborn care. We recommended review of pre-referral care for newborns to identify reasons for high fatality among this group.

6108

MORE THAN JUST AN ITCH- UNDERSTANDING ATTITUDES TO HEAD LICE TREATMENTS IN AUSTRALIAN CHILDREN AND FAMILIES

Ingrid Amgarth-Duff[1], Asha Clare Bowen[2], Bernadette Ricciardo[1], Hannah Thomas[1], Stephanie L. Enkel[3], Jacinta Walton[1], Rachel Burgess[1], Charlie Mcleod[1]

[1]The Kids Research Institute Australia, Nedlands, Australia, [2]Telethon Kids Institute, WEMBLEY, WA, Australia, [3]Nedlands, WA, Australia

Head lice affect up to one in four Australian primary school children and are associated with discomfort, embarrassment, absenteeism and financial burden. Scratching may lead to secondary bacterial infection with *Streptococcus pyogenes*, increasing risk of rheumatic fever and rheumatic heart disease among Aboriginal children in Australia. Our research found high burdens in remote (50%) and urban (18%) Aboriginal communities. Families report current topical treatments are labour-intensive, unpleasant and difficult to administer, with few alternatives available. Oral treatments may offer a simpler, more discreet option but have not been evaluated or approved in Australia. This study explores community views and priorities to inform a culturally informed, community-led clinical trial. We conducted a Delphi study and consensus workshop with adults experienced in treating head lice. Three Delphi rounds collected qualitative and quantitative data. Round 1 explore treatment burden and potential patient-reported outcome measures (PROMs). Rounds 2 and 3 involved rating PROMs on 1-5 Likert scale, with ≥70% agreement indicating consensus. A workshop discussed items that didn't reach a consensus and perspectives on oral treatments. Data was analysed descriptively and thematically. To date, 57 participants have completed Round 1 and 41 completed Round 2. Most reported treating head lice using topical products and combing. Treatment was described as repetitive, costly and disruptive, with impacts including itch, disturbed sleep and embarrassment. Oral therapy was viewed as more convenient and potentially more effective. Priority PROMs included itch relief, sleep disruption, child comfort, speed of clearance, recurrence and stigma. In conclusion, findings demonstrate that families experience head lice treatment as a substantial practical and psychosocial burden with

strong interest in oral treatments. Understanding community priorities has implications for international clinical trial design, translation into practice and improving outcomes for children with this neglected tropical disease.

6109

EFFECTS OF PRECIPITATION ON EARLY CHILDHOOD GROWTH PATTERNS IN AMHARA, ETHIOPIA

Kathleen Kurowski[1], Bezawit Mesfin Hunegnaw[2], Yahya Mohammed[3], Chalachew Bekele[3], Fisseha Tadesse[2], Clara Pons Duran[4], Delayehu Bekele[5], Lisanu Taddesse[3], Tarik Benmarhnia[6], Grace Chan[1]

[1]Children's Hospital of Philadelphia, Philadelphia, PA, United States, [2]St. Paul's Hospital of Millennium Medical College, Addis Ababa, Ethiopia, [3]HaSET Maternal and Child Health Research Program, Addis Ababa, Ethiopia, [4]Cerdanyola Del Vallès, Spain, [5]Saint Paul's Hospital Millennium Medical College, Addis Ababa-Gulele Sub City, Ethiopia, [6]University of California, San Diego, San Diego, CA, United States

In Ethiopia, the prevalence of malnutrition and growth faltering is high and continues to be a problem. Environmental factors, such as precipitation anomalies, can impact child undernutrition through food security, crop production and infectious disease risk. Understanding the long-term effects of precipitation exposures on growth patterns is essential in designing effective solutions. The objective of this study is to explore the effects of precipitation trends over time on growth outcomes and trajectories. We analyzed data from the prospective Birhan cohort study following 4354 children in Amhara, Ethiopia, between December 2018 to November 2020. Eligible participants were children aged 0 to 24 months who were enrolled in the Birhan cohort and had their length and weight measured at least twice. The primary outcomes were growth trajectories, as defined by the patterns of stunting, underweight and wasting across all visits. Quintiles of mean daily precipitation in millimeters (mm), derived from a longitudinal gridded dataset, across the 9 months preceding the last visit date were used as the main exposure. Multinomial logistic regression models were used to estimate the odds ratios (ORs) and 95% confidence intervals (CIs) between trajectory outcomes and precipitation exposure. Analysis included 2542 (58%) children out of 4354 enrolled, with at least two visits with weight and height measured. Majority of children were never underweight (73%) or never wasted (70%), while a smaller portion were never stunted (37%). Children who were underweight (14%), stunted (19%) or wasted (20%) often reversed. Increasing quintiles of mean daily precipitation over 9 months increased the odds of reversing underweight as well as wasting. Increasing quintiles of mean precipitation over 9 months decreased the odds of persisting stunting. Increasing mean daily precipitation provided some benefit in protecting children from the effects of undernutrition. Understanding climate-sensitive factors that could influence incidence of underweight, stunting and wasting in this cohort could be informative for future interventions.

6110

FLOOD EXPOSURE, WATER SANITATION AND HYGIENE (WASH); ACCESS, CHILD ILLNESS, AND MATERNAL QUALITY OF LIFE AMONG CLIMATE-DISPLACED RIVERINE ISLAND COMMUNITIES IN BANGLADESH

ABUL KASHAM MD. SHOAB[1], Md Mahbubur Rahman[1], Gabriella Rose Barratt Heitmann[2], Dr. Farjana Jahan[1], Afsana Yesmin[1], Suhi Hanif[2], Md. Sakib Hossain[1], Md. Rana Miah[1], Zahid Hayat Mahmud[1], Reza Mostary Akhter[1], Dr. Fahmida Tofail[1], Ayse Ercumen Ercumen[3], Jade Benjamin-Chung[2]

[1]International Centre for Diarrhoeal Disease Research, Bangladesh, DHAKA, Bangladesh, [2]Stanford University, Stanford, CA, United States, [3]North Carolina State Univ, Raleigh, NC, United States

Many people in Bangladesh have been displaced to riverine islands ("chars") due to climate-intensified river erosion. We compared demographics; water sanitation and hygiene (WASH); maternal quality of life (QOL); and child health between char and mainland households enrolled in the CRADLE Trial in Sirajganj, Bangladesh. We analyzed data from 800 households with pregnant women (char: N=338; mainland: N=462) recruited at 14-30 weeks gestation. Sociodemographic, WASH, and compound flooding data were collected at baseline. We assessed child diarrhea and respiratory illness at baseline, 6- and 12-month follow-up and maternal QOL (WHO QOL-Brief) at 3 and 12 months. We estimated prevalence ratios (PRs) with Poisson regression models with robust standard errors. Models for sociodemographic and WASH outcomes adjusted for maternal education, household size, paternal occupation, wealth index, animal ownership, and flooding; child health and QOL models additionally adjusted for improved sanitation, improved handwashing station, month, and study arm. Char households had lower paternal education and higher agricultural and animal ownership vs. mainland households. Char households were less likely to have latrines with a water seal (PR=0.81, 95% CI, 0.67, 0.98), but latrine ownership and handwashing did not differ. Compound flooding was higher in char vs mainland households (PR = 1.50, 95% CI: 1.18 to 1.90). Child respiratory illness prevalence was higher in char vs. mainland households (PR=1.62, 95% CI: 1.27 to 2.06); child diarrhea prevalence did not differ (PR=0.97, 95% CI: 0.76 to 1.24). Char residents reported lower QOL than mainland residents across all four QOL domains, with statistically significant deficits in the environment domain (β = -5.50, 95% CI: -7.68 to -3.32) and overall QOL (β: -1.50, 95% CI: -2.88 to -0.13), while differences in physical and social domains did not reach statistical significance. These results reveal deep disparities in socioeconomic status, well-being, and health in char communities, underscoring the urgency for tailored interventions to address structural inequities and mitigate health risks.

6111

THE DUAL BURDEN OF ANAEMIA AND STUNTING IN CHILDREN UNDER FIVE IN NIGERIA: SHARED DETERMINANTS AND PUBLIC HEALTH IMPLICATIONS

Nihinlolawa Grace Alo[1], Gayawan Ezra[2], Ademola Adetunji[1], Gabriel Oladayo Awoleye[1]

[1]Federal Polytechnic, Ile-Oluji, Nigeria, [2]Federal University of Technology, Akure, Nigeria

Anaemia and stunting remain major public health challenges affecting children under five years of age in low-and middle-income countries, particularly in sub-Saharan Africa. These conditions often coexist and share common socioeconomic, environmental, and health-related determinants, yet they are often studied independently. We investigate the dual burden of anaemia and stunting among children under five in Nigeria, focusing on their shared determinants and spatial distribution. Data were obtained from Nigeria Demographic and Health Survey and the analysis included 9,562 children under five years of age. Anaemia was defined as hemoglobin concentration below 11 g/dl, while stunting was measured using height-for-age Z-scores below-2 standard deviations according to the World Health Organization child growth standards. A Bayesian shared component model was employed to jointly examine the spatial variation of anaemia and stunting across Nigeria's 36 states while accounting for individual, household, and environmental covariates. This model decomposes spatial variation into shared and disease-specific components, enabling the identification of common underlying risk structures. The results show that children from wealthier households and those of educated mothers exhibited significantly lower odds of anaemia, stunting, and their combined occurrence. Access to improved water sources, and maternal antenatal care services demonstrated protective effects. The spatial analysis revealed pronounced geographic disparities, with a high shared burden of anaemia and stunting concentrated in several northern states, including Sokoto, Kebbi, and Borno. The findings highlight the importance of integrated and geographically targeted interventions to address the dual burden of anaemia and stunting. Policies that simultaneously strengthen maternal education, improve household socioeconomic conditions, expand access to quality health services, and enhance water, sanitation, and hygiene infrastructure are essential for reducing childhood undernutrition and improving long-term population health in Nigeria.

6112

CONTINUOUS MENTORSHIP TO IMPROVE PNEUMONIA CASE MANAGEMENT IN COMMUNITY HEALTH PROGRAMS: EVIDENCE FROM TWO STATES IN NIGERIA

Chinedu Egwuonwu[1], Gloria Sillo[1], Meredith Center[2], Todd Jennings[3], Dare Eguavon[1], Murtala Muhammad[1], Eno Angela Attah[4], Abubakar Muhammad Noma[5]

[1]PATH, Abuja, Nigeria, [2]PATH, Bozeman, MT, United States, [3]PATH, Lusaka, Zambia, [4]Akwa Ibom State Primary Health Care Development Agency, Akwa Ibom, Nigeria, [5]Kebbi State Primary Health Care Development Agency, Kebbi, Nigeria

Accurate assessment and treatment of pneumonia within integrated community case management (iCCM) programs depend on trained community health workers' (CHWs) ability to correctly measure respiratory rates to identify fast breathing in children with cough. However, acquiring this competency and then maintaining it over time remains a challenge, particularly in resource-constrained settings. The Rotary Healthy Communities Challenge (RHCC) implemented post-training mentorship to strengthen CHW adherence to pneumonia case management protocols in two Nigerian states. On-the-job mentoring was conducted during supervisory visits, and monthly data validation meetings to reinforce respiratory rate assessment and pneumonia treatment protocols. A programmatic analysis was conducted using routine iCCM service data from October 2025 to January 2026 across Akwa Ibom and Kebbi States. The primary indicator was the proportion of children under five with fast breathing treated with amoxicillin, with values above 100%, indicating overtreatment. Continuous mentorship was associated with a reduction in overtreatment for childhood pneumonia, with overall treatment rates declining from 117% to 98% within four months, though gaps persisted in Kebbi. At baseline, substantial overtreatment was observed, particularly in Kebbi, where treatment rates were 148% in October and increased to 161% in November, indicating significant treatment of children without fast breathing with amoxicillin, which is both unnecessary for the child and constitutes a risk of antimicrobial resistance. Following sustained mentorship, overall treatment rates improved to near-appropriate levels (98%) by January 2026. The RHCC project continues, and additional data will be analyzed each month to confirm that continuous, embedded mentorship within routine supervision and data review platforms can improve on-going adherence to pneumonia case management protocols. Strengthening ongoing mentorship is critical for reducing inappropriate antibiotic use and improving quality of care in community-based health programs.

6113

STRENGTHENING MATERNAL IMMUNISATION READINESS IN THE GAMBIA: LESSONS FROM THE EARLY IMPLEMENTATION OF SMART PAPER TECHNOLOGY

Oluwatosin Nkereuwem[1], Jana Prauseová[2], Rachel Ford[3], Alpha Omar Jallow[1], Beate Kampmann[2], Joanna Busza[3]

[1]MRC Gambia at LSHTM, Fajara, Gambia, [2]Charité Centre for Global Health and Institute for International Health, Charité Universitatsmedizin, Berlin, Germany, [3]London School of Hygiene & Tropical Medicine, London, United Kingdom

Effective maternal immunisation programmes depend on strong routine data systems for documentation, follow-up, and continuity of care. In many LMICs, these systems remain paper-based and fragmented. Smart Paper Technology (SPT), a hybrid paper-digital documentation system, offers lessons for strengthening the data infrastructure needed for future maternal immunisation programmes. qualitative study was conducted in two pilot facilities in the Western Region of The Gambia between September and December 2024. Data were collected using 30 semi-structured interviews, four focus group discussions with Ministry of Health staff and collaborating institutions, and non-participant observations across key maternal and newborn service areas. Data were analysed thematically using an implementation-focused approach. SPT was perceived as feasible and broadly acceptable because it built on familiar paper-based workflows while improving reporting, information retrieval, and maternal-infant record linkage. Health workers described cleaner and more complete data, with better potential for tracking service use and documenting key maternal and newborn interventions, including immunisation-related records. However, implementation was shaped by contextual constraints. Uneven training, dual documentation, limited internet connectivity, scanning errors, storage constraints, and concerns about data governance affected system use. Staff responded through peer support, remote troubleshooting, and local workarounds. In conclusion, early implementation of SPT highlights how stronger routine documentation systems can support maternal immunisation readiness through improved data quality, continuity of care, and service coordination. Although not introduced specifically for vaccination, the system offers relevant lessons for building the health information foundations needed for effective maternal immunisation delivery. Scale-up will require sustained training, clear governance, adequate infrastructure, and implementation strategies responsive to routine service realities.

6114

BURDEN AND TREATMENT OF ACUTE RESPIRATORY INFECTIONS (ARI) AND DIARRHEA AMONG CHILDREN UNDER FIVE IN BALOCHISTAN, PAKISTAN FINDINGS FROM A POPULATION-BASED HOUSEHOLD SURVEY

Sajid Bashir Soofi[1], Imtiaz Hussain[1], Muhammad Umer[1], Ahmad Khan[1], Shabina Ariff[2]

[1]Aga Khan University, Karachi, Pakistan, [2]Aga Khan University, Karachi 74800, Pakistan

Acute respiratory infections (ARI) and diarrhea remain the leading causes of morbidity and mortality among children under five in Pakistan. Balochistan, characterized by geographic and health system disparities, requires granular analysis to identify underserved populations. This study assesses the prevalence and treatment coverage of ARI and diarrhea across provincial, divisional, district, and urban-rural levels. A cross-sectional survey using a probability-based stratified two-stage cluster sampling approach was conducted among children under five. Indicators included ARI prevalence, ARI treatment coverage, diarrhea prevalence (two-week recall), and diarrhea treatment coverage. Analyses were stratified by residence and geography, with low-performing districts identified based on high burden and/or low treatment. A total of 27,584 children under five were surveyed. Provincially, ARI prevalence was 4.47% and diarrhea prevalence 15.38%, with treatment coverage of 65.64% and 78.11%, respectively. Rural children had higher ARI (4.88% vs 3.49%) and diarrhea prevalence (17.26% vs 10.81%) than urban children, while diarrhea treatment was lower in urban areas (72.89% vs 79.45%). At the divisional level, Kalat had the highest burden (ARI 10.45%, diarrhea 23.37%), while Sibi showed high diarrhea prevalence (26.71%). Mekran (24.53%) and Nasirabad (35.08%) had poor ARI treatment coverage. Districts with high burden included Killa Abdullah, Khuzdar, Washuk, and Sohbatpur. Poor treatment coverage was observed in Surab, Hub, Musakhel, Nasirabad, and Pishin, with Surab and Musakhel showing critical dual gaps. In conclusion, marked geographic disparities persist in ARI and diarrhea burden and treatment in Balochistan. Targeted, district-specific interventions are needed to improve access to timely care and reduce under-five morbidity.

6115

ASSESSING SERVICE READINESS AND QUALITY OF IMMUNIZATION DELIVERY IN PAKISTAN FINDINGS FROM FACILITY AND OUTREACH OBSERVATIONS

Muhammad Umer[1], Ahmad Khan[1], Imtiaz Hussain[1], Shabina Ariff[2], Sajid Bashir Soofi[3]

[1]Aga Khan University, Karachi, Pakistan, [2]Aga Khan University, Karachi 74800, Pakistan, [3]Aga Khan University, Pakistan, Karachi, SD, Pakistan

Improving immunization coverage requires addressing both demand-side barriers and service delivery quality. However, real-time evidence on

immunization practices in Pakistan remains limited. This study assessed the quality of routine immunization services through direct observation across multiple delivery platforms. A cross-sectional participatory observation assessment was conducted in 279 fixed vaccination sites and 279 outreach sessions across 31 districts. Districts were categorized by Penta3 coverage (low, medium, high), with stratified random selection of facilities, based on vaccination volume in 2024. Structured checklists were used to observe fixed-site (49 items, 6 domains) and outreach sessions (35 items, 8 domains), covering service readiness, infrastructure, cold chain, caregiver communication, documentation, and community engagement. Items were scored binary. overall compliance scores were calculated. Fixed-site compliance averaged 70.1%, with variation across provinces (58.8% in Balochistan to 79.6% in Islamabad). Documentation (85.4%) and cold chain (77.6%) performed well, while infrastructure (53.2%) and service availability (65.3%) lagged. Gaps included delayed session start times (47%), inadequate privacy and seating (>60%), frequent cold chain disruptions (>75%), poor hand hygiene (>90%) and skipped vaccines in over half of facilities. Caregivers communication was weak, limited awareness of vaccines, side effects, and follow-up. Outreach sessions showed slightly higher compliance (73.7%), with strong workforce capacity (86.8%) and logistics (83.1%). However, microplanning (52.3%) and community engagement (56.4%) remained weak. Poor tracking of zero-dose/defaulters (40%), and minimal IEC use (>90%), infection prevention gaps (90.6%) were noted. **Conclusions** Gaps in service readiness, infection prevention, communication, and microplanning undermine quality. Strengthening frontline practices, infrastructure and community engagement is essential to improve equitable immunization outcomes.

6116

UNDERSTANDING PERCEPTIONS AND DELAYS IN ANTENATAL, LABOR, AND POSTPARTUM CARE IN RURAL BANGLADESH

Shahana Parveen[1], Farhana Hasnat Khan[1], Dalia Yeasmin[1], Syead Tamim Mahmud[1], Maria Maixenchs[2], Shams El Arifeen[1], Emily S. Gurley[3], Mohammad Zahid Hossain[1], John Blevins[4]

[1]icddr,b, Dhaka, Bangladesh, [2]ISGlobal, Barcelona, Spain, [3]John Hopkins University, Baltimore, MD, United States, [4]Emory University, Atlanta, GA, United States

Antenatal care (ANC) reduces maternal and neonatal complications, yet utilization remains suboptimal in resource-limited settings. This study explored perceptions and care-seeking patterns across pregnancy, labor, and postpartum periods in rural Bangladesh. A qualitative rapid assessment was conducted from June to July 2024 in Baliakandi sub-district in Faridpur district. We performed 18 interviews with pregnant women, family members, traditional healers, and community members, and two focus group discussions with elderly women and medical providers. Data was analyzed thematically. Participants described pregnancy as a valued but vulnerable period. Husbands and in-laws largely determined ANC decisions, often delaying care until complications emerged. Prior adverse outcomes and trust in specific healthcare providers influenced care-seeking, while cost, distance, overcrowding facilities, and perceived low risk contributed to delay. Participants described labor often began at home, with reliance on practices such as blessed water, oil massage, and herbal remedies by traditional birth attendants (TBA) or healers. Facility care was sought when complications arose or TBAs referred. When public services were unreliable, women shifted to private facilities. Families often delayed in reaching facilities, and providers got limited time to share clinical information. All these contributed to delayed treatment and adverse outcomes, including stillbirth. Women preferred vaginal delivery but families accepted cesarean section in emergencies. Cesarean decisions were sometimes made beforehand if there was a previous cesarean or providers identified risk. For postpartum and newborn care, women and families relied on female relatives, while expressing dissatisfaction with facility care due to insufficient services. Our findings indicate that delays in care-seeking are driven by family perceptions, sociocultural norms, and health system gaps. Interventions should promote women's involvement in decision-making for timely care-seeking alongside improving the quality of public healthcare facility services.

6117

MAPPING INTRAPARTUM COMPLICATION AND NEWBORN RESUSCITATION IN A RURAL SUB-DISTRICT OF BANGLADESH

Mohammad Zahid Hossain[1], Rajib Biswas[1], Taukir Tanjim[1], Sabbit Khan[1], Atique Iqbal Chowdhury[1], Asraful Alam[1], Afsana Afrin[1], Afruna Rahman[1], Muntasir Alam[1], Shahana Parveen[1], Qazi Sadeq-ur Rahman[1], Shams El Arifeen[1], Emily S. Gurley[2]

[1]icddr,b, Dhaka, Bangladesh, [2]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

In Bangladesh, intrapartum complications significantly contribute to neonatal mortality, with a rate of 16 per 1,000 live births in 2022. Despite increased facility deliveries, newborn care quality, especially resuscitation, remains suboptimal. This study aimed to assess the frequency of intrapartum complications and resuscitation interventions for compromised newborns with no cry or breathing difficulties at birth in a rural subdistrict. We analyzed pregnancy surveillance data from the Child Health and Mortality Prevention Surveillance (CHAMPS) in Baliakandi sub-district, Rajbari (2020–2024), including 21,318 live births. Intrapartum complication, birth compromise and care practices were obtained through postnatal maternal interviews. Regression-based models and mediation analysis with bootstrapping were performed to explore the extent to which birth compromise mediates the association between intrapartum complications, resuscitation and neonatal mortality. Overall, 33% of mothers reported at least one intrapartum complication, with the most common being obstructed labor (13%), ruptured membrane (8%), oligohydramnios (7%), reduced fetal movement (5%), and prolonged labor (5%). Birth compromise was observed in 10% (2,201/21,318) of newborns. Mediation analysis showed that birth compromise accounted for 37% of the effect of intrapartum complications on early neonatal mortality. Among compromised newborns, 88% received stimulation, 48% supplementary oxygen, 15% suction, 5% bag-mask ventilation, and 1% CPAP. Early neonatal mortality in this group was 9%. Preterm birth (adjusted OR 3.56, 95% CI 2.60–4.88) were strongly associated with higher mortality. Despite high use of basic interventions, access to effective ventilation remained limited. At the policy level, the results highlight the need for national strategies to strengthen intrapartum and immediate newborn care, including availability of bag-mask and CPAP equipment.

6118

A REPLICABLE MODEL FOR COST-EFFECTIVE AND SUSTAINABLE KNEE REPLACEMENTS IN CAMEROON, AFRICA

Akankcha Alok[1], Marie Alixe Kima[2], Peter Kima[1]

[1]University of Florida, Gainesville, FL, United States, [2]Cayhiti, inc, Gainesville, FL, United States

This project was formulated to address the lack of facilities for total knee arthroplasties in Cameroon, Africa. Most patients had no locally available options and only a few who could afford it would travel abroad to get their procedures done. Two campaigns were organized at the Douala General Hospital (DGH), Cameroon, by non-profit organizations (NGOs) Cayhiti, inc, and Walkstrong/Signature Orthopedics, USA, to design an affordable arthroplasty program that meets the needs of the local population in a sustainable and cost-efficient manner. A robust interactive exchange was instituted between surgeons in Cameroon and USA to individually discuss patients recruited at DGH for this project. Patient demographics, clinical data, X-rays, and BMI status were evaluated. The most common indications for knee replacement were osteoarthritis and valgus deformities. Protocols for achieving adequate infection control with MRSA screen and addition of vancomycin if positive, chlorhexidine bath, mupirocin to all nares and peri-operative cefuroxime with complexity dependent dose, pain management without opioids and steroids and extensive post-surgery physical therapy protocols were developed. Successful procedures were performed on 30 patients with an average age of 62.2, average BMI of 34.4, including 22 females, 8 males, 9 rights, 15 lefts and 6 bilateral knee replacements. There

was only one infection noted in a patient with a newly diagnosed gout of the replaced joint at 12-month follow-up. Three local physicians from DGH were trained to facilitate replication of these measures. Effective completion of the project was accomplished with a markedly reduced cost which was less than half the price of the procedure in the USA and with minimal complications. The trained local physicians now perform the procedures successfully for the previously underserved population and continue to receive high quality instrument supplies at a reduced cost from the NGOs. This is a unique project that has been executed in Cameroon for the first time. It serves as an illustrative example that can be replicated in other resource-poor areas of the world for people in need.

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THE INFLUENCE OF UNIVERSITY CURRICULUM STRUCTURE ON STUDENTS MENTAL HEALTH IN CAMEROON

Andreas Gara Tawum

University of Yaoundé 1, WOHEP GSC-Yaounde and Centre Pasteur du Cameroun, Yaounde, Cameroon

Universities influence both academic achievement and psychological development. The transition to higher education occurs during a life stage marked by vulnerability to stress, anxiety and depression. Student mental health concerns are rising in Cameroon, yet the role of university curriculum structure remains under-researched. This study investigated the influence of university curriculum structure on student mental health in Cameroonian public universities. An explanatory sequential mixed-methods design was employed. Quantitative data were collected from 550 students in eight state universities using a structured questionnaire assessing course load, evaluation strategies, scheduling patterns, progression policies, mentorship programmes and mental health indicators (stress, anxiety, depression and well-being). Qualitative data was also collected from 12 lecturers using semi-structured interview guide. Data were analysed using descriptive statistics, Spearman rank correlation, Kruskal-Wallis and Mann-Whitney tests and linear regression. Qualitative findings enriched interpretation of quantitative results. Significant differences in mental health were found across age, gender, socio-economic status (SES) and level of study ($p < .001$). Low SES, female gender and undergraduate status were associated with higher mental health risk. Strong positive correlations were observed between mental health challenges and course load ($r = 0.708$), evaluation strategies ($r = 0.658$), progression policies ($r = 0.657$) and scheduling ($r = 0.569$). Curriculum related variables significantly predicted mental health outcomes after controlling for demographics. Mentorship programmes appeared insufficient to moderate academic stress. Curriculum structure substantially shapes student mental health in Cameroon, underscoring the need for reduced course load, balanced assessments practices, flexible semester scheduling, supportive progression policies and stronger mentorship programs.

6120

ENVIRONMENTAL DRIVERS OF PEDIATRIC POSTINFECTIOUS HYDROCEPHALUS (PIH) IN WESTERN UGANDA: CASE-CONTROL STUDY

Mehal Churiwal[1], Claire Baudler[2], Timothy Nyanzi[3], Patrick Hughes[4], David Kitya[3], Edgar Mulogo[3], Ross M. Boyce[2]

[1]Fulbright Program, Washington, DC, United States, [2]University of North Carolina at Chapel Hill, Chapel Hill, NC, United States, [3]Mbarara University of Science and Technology, Mbarara, Uganda, [4]Durham University, Durham, United Kingdom

Post-infectious hydrocephalus (PIH) is a leading driver of neurosurgical intervention among children in sub-Saharan Africa. PIH is frequently preceded by neonatal sepsis, yet environmental and early-life risk factors are poorly defined. We conducted a matched case-control study in western Uganda to evaluate peri-domestic and perinatal factors associated with PIH. Cases included children < 8 years diagnosed with PIH between January 2021 and December 2023, with two age- and village-matched controls per case. Caregivers completed structured questionnaires documenting perinatal/postnatal exposures. A total of 154 children were enrolled (64 cases, 90 controls). Among the assessed environmental factors, agricultural land immediately adjacent to the household was associated with PIH (51% vs. 29%, p=0.02). Several early-life exposures demonstrated strong associations with PIH. including admission to a hospital shortly after birth (27% vs. 2%, p<0.0001) and exposure to antibiotics or medications at the time of birth (21% vs. 1%, p=0.0004). Traditional postnatal practices were also more frequently reported among cases (12% vs. 2%, p=0.002), suggesting a potential modifiable risk factor rather than a proxy for underlying illness. Additionally, fewer cases reported consistent feeding with milk or porridge during early infancy (63% vs. 92%, p<0.0001), though this may reflect either a causal relationship or reduced feeding in the setting of early illness. These findings suggest that early neonatal exposures—particularly healthcare-associated and postnatal practices—may be critical drivers warranting further investigation, while peri-domestic environmental factors play a more limited role in PIH risk, except possibly for agricultural proximity. Identifying interventions that target infection prevention in neonatal care settings and promote safer postnatal practices may reduce the burden of PIH in similar settings.

6121

CO-CREATING HEALTH RESEARCH CAPABILITIES IN LOW- AND MIDDLE-INCOME COUNTRIES: LESSONS FROM INDUSTRY-LED INITIATIVES

Akiko N. Keller[1], Jenni Gray[1], Chanelle Pillay[2], Jonathan M. Spector[3], Sandra D. Felix[4], **Paul G. Ashigbie**[3]

[1]Global Health, Novartis, Basel, Switzerland, [2]Global Health, Novartis, Johannesburg, South Africa, [3]Biomedical Research, Novartis, Cambridge, MA, United States, [4]Translational Medicine, Novartis, Basel, Switzerland

Research is fundamental to advancing the prevention, diagnosis, and treatment of disease. Yet progress in addressing unmet health needs remains constrained by inadequate research capabilities in low- and middle-income countries (LMICs), which shoulder a disproportionate share of the global burden of disease. Over the past two decades, Novartis together with partner institutions, has implemented a diverse portfolio of health research capability strengthening (HRCS) initiatives in LMICs. Here we characterize the scope of these initiatives and examine the factors underpinning their effectiveness and impact. Data were derived from program documentation, internal records, and monitoring and evaluation feedback from trainees. The HRCS initiatives span a broad range of activities: scientific and professional development training delivered through workshops, hands-on projects, and mentorship; and equipment donations. These programs include tailored, short-term technical support addressing acute needs, and long-standing capability-strengthening efforts. Beneficiaries include students, early-career and advanced professionals, as well as research institutions across Africa, Asia, and Latin America. Initiatives are implemented within robust governance frameworks, supported by formal partnership agreements that articulate a shared vision and clearly define the roles and responsibilities of each partner. Local ownership has been a defining feature, with programs designed to address the expressed needs and priorities of trainees and their institutions. A central element of these initiatives is bi-directional knowledge exchange: while participants benefit from Novartis' scientific expertise and resources, Novartis scientists gain critical insights into disease populations and health systems. Routine evaluation and cross-program knowledge sharing are integral components, enabling continuous learning, quality improvement, and adaptation to evolving needs. This case study highlights the unique and complementary role that industry can play in building locally anchored research capabilities in LMICs.

6122

PLACING HEALTH EQUITY AT THE CENTER OF GLOBAL HEALTH EDUCATION: AN EASILY IMPLEMENTABLE GLOBAL/LOCAL CURRICULUM

Gaia Linfield[1], Radhika Lu Sundararajan[2], Laura Greisman[2]
[1]New York Presbyterian-Weill Cornell, New York, NY, United States, [2]Weill Cornell Medicine, New York, NY, United States

Growing awareness of global health's colonial roots has shifted education from exclusively focusing on short-term international experiences toward engagement with local underserved communities. This "global/local" framework emphasizes that the skills to care for vulnerable populations can be globally transferable. Despite rising interest, few descriptions of global/local curricula exist in undergraduate medical education (UME). We revitalized an optional firstyear global/local course to focus on health equity and the application of global principles locally while preserving student flexibility and minimizing faculty burden. The curriculum included a virtual orientation and six weeks of selfdirected learning using curated texts, podcasts, reflection prompts organized around six themes: (1) Global/Local Health Disparities; (2) Social and Structural Drivers; (3) Health Insurance; (4) Language Discordance; (5) Immigrant and Refugee Health; and (6) Health as a Human Right. Students were encouraged to participate in community volunteer opportunities, submitted a final reflection essay, and participated in a facilitated reflection session with global health faculty and nearpeers. From Spring 2023-2025, 30 students participated; 27 completed an anonymous threeitem postsurvey. Twentytwo respondents agreed the course improved their understanding of social/structural drivers of health and global/local equity. Students valued the course's flexibility, selfpaced format, and opportunities for peer learning. Qualitative analysis of reflection essays is ongoing. This curriculum reflects postCOVID educational priorities: asynchronous, selfdirected learning; student autonomy; and low cost and administrative burden. Involving students in syllabus updates and recruiting nearpeer facilitators supported ongoing adaptation and collaboration. According to participant feedback, the course helped learners recognize that meaningful global health work begins locally and is grounded in health equity. This model offers an accessible, easily adaptable approach for other institutions.

6123

MAXIMUM TEMPERATURE-INDUCED NEONATAL MORTALITY AND INEQUITIES IN RELATIVE VULNERABILITIES IN GHANA: A DISTRIBUTED LAG PANEL ANALYSIS WITH COUNTERFACTUAL RISK ESTIMATION; 2008-2025.

Duah Dwomoh[1], Monica Baaba Jones[2], **Frances Bafua Otoo-Kati**[1], Ama Frimpomaa Boateng[2], Mawuli Dzodzomenyo[1]
[1]School of Public Health, University of Ghana, Legon, Accra, Ghana, [2]University of Ghana Medical Centre, University of Ghana, Legon, Accra, Ghana

Climate change imperils maternal, neonatal, and child health, yet evidence on implications of temperature-induced spikes on neonatal mortality in sub-Saharan Africa is scanty. Perceiving how sustained, prolonged heat exposure contributes to neonatal deaths is dire to inform and develop effective climate adaptation strategies and strengthen health-system resilience in vulnerable areas. We conducted a comprehensive longitudinal district-level panel analysis using robust statistical models to quantify the impact of varying maximum monthly temperature on neonatal mortality and to identify high-risk burden communities across 261 administrative districts. We integrated two distinct detailed monthly data sources on health and climate spanning 2008-2025. Analysis used advanced distributed lag regression models that incorporated district fixed effects, seasonal adjustments and rainfall controls. Neonatal mortality rates were modelled using findings based on Poisson, negative binomial and quasi-Poisson models with deliveries as the exposure. We also generated counterfactual risk estimates under a median-maximum temperature scenario. The data showed a correlation between higher maximum monthly temperatures and increased neonatal mortality in Ghana. A one-unit increase in the combined maximum temperatures at lags 0-3 months is linked to a 9% rise in neonatal mortality rates (incidence rate ratio 1.09, 95% CI 1.04 to 1.13). From 2008-2025, approximately 652 [95% CI: 516 to 788] neonatal deaths can be directly linked to exposure to temperatures above the median (28.2°C). The impact of temperature on neonatal mortality varied greatly across districts and regions, with the highest risk seen in Ghana's five northern regions, where median monthly temperatures consistently exceed the national average. Expediently, heat-adaptive interventions such as enhanced neonatal monitoring and early-warning systems should be deployed during hot months, focusing efforts on high-exposure districts and regions to reduce temperature-related neonatal fatalities and save lives.

6124

THE PHYSICAL AND SYSTEMIC COST OF CARE: A NATIONAL STUDY OF OCCUPATIONAL HAZARDS AND MIGRATION INTENT AMONG 776 NIGERIAN PHYSICIANS

Alamin Muhammad Usman[1], Aisha Suleiman[1], Musa Yahaya[2]
[1]Dalhatu Araf Specialist Hospital , Nigeria., Lafia, Nasarawa state, Nigeria, [2]University Hospitals Birmingham NHS Trust, Birmingham, United Kingdom

For years, the conversation around the Nigerian medical "brain drain" has focused on salaries. However, the migration crisis is fueled by a deeper, more visceral reality, the physical and reproductive hazards facing those who remain. This national, cross-sectional mixed-methods study of 776 Nigerian doctors across all professional ranks aimed to quantify the physiological and physical toll of current labor policies and the absence of working hours regulations. The mean post-call fatigue score was 8.15 plus or minus 2.19. Chronic morbidity was nearly universal, with stress-related headaches (81.8%) and mental health issues including anxiety and depression (70.1%) being the most prevalent. Acute, life-threatening occupational hazards were also common: fainting at work (14.8%), fatigue-related road traffic accidents (11.3%), and work-linked miscarriages (8.6%). Female physicians reported significantly higher fatigue than males ($p<0.001$). Professional rank was a significant factor ($p<0.001$), with house officers and registrars bearing the highest burden. A near-universal intent to migrate (94.7%) was recorded, driven by inadequate compensation (95.2%) and hazardous conditions. The current "alternate-day" call system has transitioned from a professional challenge into a physical hazard that threatens the lives of both doctors and patients. To stem the migration intent and preserve the collapsing healthcare workforce, the Nigerian government must move beyond purely fiscal interventions and legislate a "Physician Safety Act." We urgently recommend a mandatory 24-hour rest period following 24-hour shifts, the immediate regulation of maximum weekly work hours, and the implementation of protected duty hours for pregnant and nursing physicians to prevent further reproductive and physical trauma.

6125

AN INTEGRATED MODEL FOR GLOBAL HEALTH COLLABORATION: A DESCRIPTION OF THE DUKE-MOI PARTNERSHIP

Edna Sang[1], Joseph Kipkoech Kirui[2], Titus G. Ngeno[2], Dennis Sang[3], Winstone Nyandiko[3], Julia Songok[4], David Lagat[4], Felix Barasa[5], Eve S. Puffer[2], Gerald S. Bloomfield[2], Wendy P. O'Meara[2]
[1]Duke Global Inc, Eldoret, Kenya, [2]Duke University, Durham, NC, United States, [3]Academic Model Providing Access To Healthcare, Eldoret, Kenya, [4]Moi University School of Medicine, Eldoret, Kenya, [5]Moi Teaching and Referral Hospital, Eldoret, Kenya

Guided by a shared vision of advancing global health equity through locally grounded collaboration, the partnership between Duke Global Health Institute at Duke University and Moi University (MU)/Moi Teaching and Referral Hospital (MTRH) has operated for over two decades within the AMPATH consortium. Since 2006, the partnership has expanded from cardiology to include infectious disease, mental health, and nursing, aligning with the tripartite mission of education, research, and specialized clinical care. We conducted a descriptive program evaluation to characterize

the activities and outcomes of a 20-year global health partnership. To date, 143 bidirectional rotations have occurred: 31 Duke Global Health Pathway fellows (9-12 months at MTRH); 45 Duke residents (8-week electives at MTRH); and 67 Moi trainees (38 students, 15 registrars, 14 fellows) completing clinical and research attachments at Duke. Over 50 Duke graduate students completed field-based research in Kenya. Through the Visiting Scholars program, Moi faculty received Duke-based training in cardiology, psychology, and research administration. The Moi/MTRH's Research and Sponsored Projects Office has managed over $4 million in extramural and core funding since 2013, producing over 300 co-authored publications. Local capacity building includes clinical microscopy training of over 150 laboratory technologists in Western and Northern Kenya, supported by the Partnership for Education and Academic Research Laboratory. The Tuko Pamoja Family program partnered with church congregations and trained lay community members to deliver family-based mental health interventions. The partnership established the region's first 10-bed cardiac care unit and trained seven cardiology fellows, expanding specialized care nationwide. The partnership also leverages East African networks to strengthen governance and scale research and training standards across neighboring countries. Sustained, bidirectional collaboration grounded in trust strengthens health systems, promotes equity, and supports world-class research centered on shared priorities.

6126

BACTERIAL ENHANCED SEQUENCING FOR SEPSIS TRIAGE (BESST): ENABLING FIELD-FORWARD DETECTION OF BACTERIA IN BLOOD TO IMPROVE SEPTICEMIA OUTCOMES

Ghyssella E. Valdiviezo[1], Andrea Luquette[1], Maren C. Fitzpatrick[1], Chunsheng Huang[1], Cameron Vincent Sayer[2], Regina Zing Cer[3], Francisco Malagon[3], Kimberly Bishop-Lilly[4]

[1]BDRD, Frederick, MD, United States, [2]Cameron Sayer, Silver Spring, MD, United States, [3]Naval Medical Research Center, Frederick, MD, United States, [4]Naval Medical Research Center, Fort Detrick, MD, United States

Sepsis is a major global public health threat that can lead to severe morbidity and mortality. It is an abnormal condition characterized by an overwhelming systemic inflammatory and immune response to microbial invasion and other stresses. Initial localized infections trigger a complex cascade involving the widespread release of inflammatory cytokines which, in a dysregulated response, begin to damage the body's own tissues progressively and could ultimately induce septic shock. For these reasons, sepsis is considered a medical emergency requiring rapid diagnosis and treatment. In the military setting, sepsis is a major cause of mortality among infectious disease cases, primarily because of the high fatality rate among septic shock occurrences. To support early diagnostic efforts in the military, the Biological Defense Research Directorate at the Naval Medical Research Command is working to develop a fast, field forward sequencing protocol for suspected septicemia using the small footprint of Oxford Nanopore Technologies platform. To increase the sensitivity of microbial DNA detection, we spiked a total of 24 human blood samples with dilutions of *E. coli*, *S. aureus*, *P. aeruginosa*, and *Plasmodium falciparum* covering a 100,000 fold range. DNA was extracted from each sample, and aliquots of the extracts were prepared using the NEB Microbiome DNA Enrichment kit for host removal. Both the original extracted DNA and the host depleted samples were used to prepare sequencing libraries that were sequenced using the short read Illumina MiSeq i100 plus platform as well as the long read ONT PromethION platform. For all microbial species, the improvement between host removal sample depth and the depth of coverage without host removal was between four and sixfold on average. Our data indicates that this commercial off the shelf approach is effective for sepsis samples and that various microbes perform differently under host depletion treatment. Implementing simple procedural methodologies such as this that increase bacterial detection sensitivity from blood in remote austere locations may help decrease mortality rates in sepsis cases.

6127

EVALUATIONS OF CLIMATE-INFORMED EARLY WARNING SYSTEMS FOR FOODBORNE, WATERBORNE, AND VECTOR-BORNE DISEASES: A SCOPING REVIEW

Maya M. Rao, Katherine O'Brien, Afrin Iqbal, Sarah E. Dumas, Timothy H. Holtz

The George Washington University, Washington, DC, United States

Climate change is altering the risk of infectious diseases with increasingly negative consequences for human health. Anticipating outbreaks before they occur and implementing adaptive measures can reduce the burden of climate-sensitive infectious diseases (CSIDs). Integrating climate variables into early warning systems can enhance outbreak prediction and provide critical information for public health systems to adapt to the changing risk of infectious diseases. While extensive research has advanced predictive models with potential applications to Climate-Informed Early Warning Systems (CIEWS), evidence of the effectiveness of CIEWS once operationalized has not been well described. We conducted a scoping review to examine evidence from evaluations of operational CIEWS for foodborne, waterborne, and vector-borne diseases. Following the PRISMA-ScR guidelines, we developed and applied a search strategy across three databases: Global Index Medicus, Scopus, and PubMed. The search string employed a combination of keywords, including climate variables, disease-related terms, early warning terms, and evaluation terms. Of the 9,915 abstracts screened, 156 met eligibility for full text review, with studies conducted in Mexico, Ethiopia, Singapore, Pakistan, Colombia, among other locations. After reviewing full texts against eligibility criteria, included articles underwent data extraction, covering the study context, target diseases, CIEWS duration in use, modeling approaches, and evaluation criteria and results. This talk will synthesize data from articles included in the review, focusing on opportunities and challenges in operationalizing CIEWS and recommendations to enhance uptake and effectiveness. We will summarize evaluation and modelling approaches employed across studies to highlight research gaps for further inquiry. This review addresses the issue of translating research into action for evidence-based climate adaptation across multiple diseases and global contexts and offers insights to enhance global CSID surveillance and guide future implementation research.

6128

MAPPING *AEDES* MOSQUITO PRESENCE AND ITS LINK TO *AEDES*-BORNE DISEASES RISK: A COMPARATIVE STUDY BETWEEN TROPICAL HIGHLANDS COUNTRIES OF THE ANDES AND THE HIMALAYAS

Adriana Urrutia[1], Daniel M. Parker[2]

[1]University of California Irvine, Irvine, CA, United States, [2]University of California, Irvine, Irvine, CA, United States

The geographic expansion of *Aedes* mosquitoes, mostly, *Ae. aegypti* and *Ae. albopictus,* has been associated with a sharp increase of *Aedes*-borne diseases in the XXI century(1). Within the current evidence landscape, a growing body of entomological initiatives is extending the analysis of mosquito range shifts beyond longitudinal and latitudinal dimensions by exploring its vertical expansion to higher elevations(2,3). Epidemiological efforts are underway globally to document diseases cases, yet data gaps have been observed (4). Despite current advances, approaches have not necessarily integrated Aedes vector data and Aedes-borne diseases data, affecting the understanding of the spatial dynamics of outbreaks. To better understand these spatial patterns, we argue that aggregating data within topographic regions is necessary to assess geographic gaps. Our study seeks to fill gaps for countries of the Andes and the Himalayas- considered topographic regions highly vulnerable to climate change. We will conduct a two-part scoping review. First, we will map recorded data on the presence of the vector in mountainous Andean (Venezuela, Colombia, Ecuador, Peru, Bolivia, Chile and Argentina) and Himalayan countries (Pakistan, Bhutan, Nepal and India). Second, we will map reported cases of Aedes-borne diseases in these settings. A search strategy will be conducted in PubMed and other databases using a combination of Medical Subject

Headings (MeSH) and free-text keywords following Arksey and O'Malley methodology for Scoping reviews(5). Boolean operators (AND/OR) will be used to combine synonyms and thematic concepts. Given the publication lag, interviews with decision-makers will be conducted to gather first-hand data on noticed elevation shifts of the vector and diseases cases. We initially found 6113 articles, with 1308 finally meeting our inclusion criteria. Preliminary findings suggest that an interaction of climatic and social factors driving the elevational expansion of Aedes-borne disease risk, while also revealing fragmented research approaches. Final results will be available by October after completion of data processing.

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COMMUNITY PERCEPTIONS OF WASTE MANAGEMENT AND MOSQUITO-BORNE ILLNESS IN MAKASSAR, INDONESIA

Morgan S. Tarpenning[1], Fakhran F. Akhyar [2], Najwati Anggraeni[2], Aliyyah Ansariadi[2], Kavita D. Coombe[1], Jumriani Ansar[2], Ansariadi Ansariadi[2], Joelle I. Rosser[1]

[1]Stanford University, Stanford, CA, United States, [2]Hasanuddin University, Makassar, Indonesia

Environmental trash pollution poses a growing threat to public health globally, especially in tropical climates such as Indonesia where trash serves as a breeding ground for disease vectors. Makassar, the largest city in Eastern Indonesia, faces a dual crisis of high dengue prevalence and widespread trash accumulation. Obtaining an understanding of trash distribution, management systems, and community perceptions is critical to designing effective, community-supported solutions. We conducted 30 in-depth interviews in the Tallo sub-district of Makassar from a parent cohort based out of Hasanuddin University. Participants were randomly selected from those who reported less frequent household trash collection. Interviews were conducted in Bahasa Indonesia or Makassarese. Interview topics covered trash management practices, knowledge of health risks, seasonal challenges, and potential solutions. Interviews were audio-recorded, transcribed, translated into English, and analyzed with a thematic approach. Interviews revealed inconsistent trash collection with disruptions due to weather, vehicle issues, and payment delays. This led many households to adopt alternative disposal methods like burning or dumping. Concern for children's health was a prominent theme, with participants linking trash to increased disease risks including dengue. Key non-health impacts included unpleasant odors, blocked drainage, aesthetics, and disrupted daily activities. Community cleanups were motivated by flood prevention and faced inconsistent participation. Potential solutions suggested included public awareness, trash bins, and more frequent collection, though entrenched behaviors and lack of disposal options were identified as barriers. These findings call for interventions that pair upstream infrastructure improvements with community-level knowledge dissemination and behavior change, leveraging existing cleanup events and neighborhood leadership structures as potential entry points for dengue prevention in Tallo and similar urban contexts.

6130

CLIMATE VARIABILITY AND MALARIA TRENDS IN RURAL TANZANIA: INSIGHTS FROM HEALTH DATA, ENVIRONMENTAL RECORDS, AND COMMUNITY NARRATIVES

Winifrida Paschal Mponzi, Dickson S. Msaky, Emmanuel W. Kaindoa

Ifakara Health Institute, Morogoro, United Republic of Tanzania

Tanzania is experiencing increasing climate variability, including changing rainfall patterns and rising temperatures, which may influence malaria transmission. Evidence linking climate variability to malaria trends at local scales remains inconsistent, particularly in areas with ongoing control interventions. This study examines whether recent malaria trends in Ifakara can be explained by climate factors alone or by a broader set of environmental and socioeconomic drivers. A mixed-methods design combined retrospective malaria data from 3 health facilities (2018-2024) with rainfall and temperature records from nearby meteorological stations. Quantitative data were analyzed to assess temporal trends and associations between climate variables and malaria cases. Qualitative data were collected from community members and analyzed thematically to explore perceptions of malaria trends and climate variability. A total of 114,460 patient records were analyzed, with a malaria test positivity rate of 9%. Malaria burden fluctuated from 2018 - 2020, declined sharply in 2021, and remained relatively low from 2022 - 2024 with minor seasonal peaks. Rainfall was highly variable and showed a weak and inconsistent temporal relationship with malaria cases, with occasional increases following periods of higher rainfall, suggesting that rainfall alone does not fully explain the observed trends. Temperature followed typical seasonal patterns but showed no clear association with malaria cases. Qualitative findings indicated a perceived decline in malaria, attributed to improved awareness, increased uptake of preventive measures, and environmental changes, alongside persistent socioeconomic and ecological factors, including poverty and local habitat conditions. Malaria trends in Ifakara cannot be explained by climate variability alone but reflect a complex interplay of climatic, environmental, and socioeconomic drivers. These findings highlight the need for integrated surveillance approaches that combine environmental, epidemiological, and community-level data to inform adaptive and targeted malaria control strategies.

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DETERMINANTS OF PERFORMANCE IN DENGUE AGE-STRUCTURE PREDICTIONS FROM SURVEILLANCE DATA

Juan Dent Hulse, Amy Wesolowski, Saki Takahashi, Derek Cummings

Johns Hopkins University, Baltimore, MD, United States

The introduction and epidemic of Zika in Brazil between 2014 and 2016 coincided with a shift in the age structure of suspected dengue cases reported to the national surveillance system (SINAN). This shift might reflect the misclassification of Zika cases or immunological interactions between these viruses. To assess whether such systematic shifts in dengue transmission patterns can be detected and used in routine surveillance, we evaluated the contribution of factors to changes in age distributions of dengue cases over time in Brazil. Using data from 2000 to 2016, we compared the observed case age distributions with predictions from age-group-specific rates pooled from the preceding five years, excluding years with fewer than 1000 cases in any age group. We modeled the log of annual deviance (a measure of fit) as a univariate linear function of year, state, dengue and Zika incidence, measures of stability in the pooled rates, and microcephaly incidence across various lags. Interactions between region (e.g., Northeast) and year, Zika, and microcephaly were also explored. There were 16,034,816 suspected cases across 24 states and 1 federal district reported to SINAN between 2000 and 2016 with sufficient annual counts and complete age and state data. We observed distributional differences in model performance between years with and without reported Zika cases only when stratified by region: deviance for years with reported Zika cases was 3.2 times larger in the Northeast (95% CI: 1.5, 6.8) and 2 times larger in the Southeast (95% CI: 0.69, 6.44) compared to years with no Zika cases reported. Higher microcephaly rates were associated with greater deviance, with evidence of a nonlinear relationship ($p < 0.02$). We further observed positive associations ($p<0.01$) between deviance and annual and historical dengue incidence, state, and measures of stability in the pooled rates. We evaluated factors contributing to deviations from the expected age distribution of suspected dengue cases using surveillance data. These findings offer a framework for detecting shifts in the age structure of reported cases, including those linked to the Zika epidemic.

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LESSONS LEARNED FROM NON-*AEDES*-BORNE ARBOVIRUSES FOR FUTURE EPIDEMIC AND PANDEMIC PREPAREDNESS: A SCOPING REVIEW OF GLOBAL LITERATURE

Ida Stevia Diget[1], Abdul-Aziz Seidu[1], Maxine Anne Whittaker[1], Tammy Allen[1], Diana P. Rojas[2], Oluwatosin Akande[2], Ingrid Brigitte Rabe[2], Sarah Holderness[2], **Maria Eugenia Castellanos Reynosa**[1]

[1]James Cook University, Douglas, Australia, [2]World Health Organisation, Geneva, Switzerland

Worldwide, non*Aedes* viruses are frequently overlooked due to the high burden of *Aedes*-borne viruses, though they are gaining recognition as major health, economic, and social threats. These viruses are strongly influenced by the human-animal interface. This can result in disproportionate impacts on rural and livestockdependent communities, where close and frequent interactions with animals and vectors are common. Understanding and disseminating lessons from these outbreaks can provide critical guidance for strengthening epidemic and pandemic preparedness and response strategies for future arbovirus outbreaks. This scoping review aimed to synthesise the existing literature on lessons learned from non-*Aedes* arbovirus epidemics. The review followed the Joanna Briggs Institute methodology and the PRISMA guidelines. It focused on peer-reviewed and grey literature written in English or Spanish, published between 2000 and 2025, with no geographic limitations. A total of 28 arboviruses were included. The review included 189 sources. West Nile virus (n=49), Rift Valley Fever Virus (n=44), Crimean-Congo haemorrhagic fever (n=37), and Japanese Encephalitis virus (n=17) were most frequently represented. Lessons learnt were extracted systematically, analysed thematically, and synthesised into a series of recommendations. These were presented in a phased approach, spanning preparedness, response, and resilience activities. A strong evidence base documented the benefits of a One Health approach for responding to non-*Aedes*-borne arboviral threats. Central to the One Health approach is collaboration and coordination among the human, animal, and environmental sectors, including representation from government officials, researchers, community leaders, and national and international stakeholders. Integration of animal, ecosystems, and human surveillance systems is essential for early detection. Further, the reviewed literature highlights both challenges and successful strategies for controlling non-mosquito viruses, which, in some cases, differ uniquely from those for controlling mosquito vectors.

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A ONE HEALTH ASSESSMENT OF MENTAL HEALTH IN THE ROHINGYA REFUGEE CAMPS

Sarah Gibes[1], Madison Kurth[1], Olivia Starich[1], Nurul Islam[1], John Uelmen[2]

[1]University of Wisconsin-Madison, Madison, WI, United States, [2]Madison, WI, United States

Over one million Rohingya refugees live in Cox's Bazar, Bangladesh, where the burden of mental health conditions is high, and access to services remains limited. Research in humanitarian settings is often hampered by a lack of community trust, cultural disconnect, and fragmented coordination across sectors. Even less attention has been given to building partnerships before data collection. Community-engaged approaches grounded in a One Health framework may strengthen the ethical and operational foundations of research with displaced populations. We are conducting a structured, pre-fieldwork planning phase to inform a forthcoming mixed-methods study on mental health service access among Rohingya refugees. Guided by a One Health framework, this phase includes stakeholder mapping across health, social, and community systems and the identification of key actors, including non-governmental organizations, community health workers, research organizations, and camp-based volunteers. We are iteratively developing a semi-structured interview guide for healthcare workers, incorporating input from local partners to ensure cultural and contextual relevance. In-country qualitative fieldwork is planned for Summer 2026. This planning phase has provided actionable insights. Early engagement highlights the importance of trust-building and ongoing collaboration with community-based actors. Stakeholder mapping emphasizes the crucial role of community health workers and local organizations in supporting culturally appropriate engagement. Considering structural constraints within camp settings has led to adjustments in recruitment and interview procedures. Iterative refinement of study instruments has focused on aligning mental health concepts with locally meaningful expressions of distress. This approach shows how community-engaged planning can improve the relevance, feasibility, and ethical rigor of research in humanitarian settings.

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WASTEWATER SURVEILLANCE FOR MONKEYPOX VIRUS IN KENYA-A PILOT STUDY

Naomi Lucchi[1], Melvin Ochieng[2], Loicer Odhiambo[2], Rosemary Nzunza[2], Eva Mwai[3], Joanne Hassan[2], Henry Mwazighe[3], Caroline Ochieng[2], Juma Bonventure[1], Samoel Khamadi[2], Jonas Hines[4]

[1]Centers for Disease Control and Prevention, Nairobi, Kenya, [2]Kenya Medical Research Institute, Nairobi, Kenya, [3]North Star Alliance, Nairobi, Kenya, [4]Centers for Disease Control and Prevention, Atlanta, GA, United States

Wastewater surveillance (WWS) has emerged as a complementary approach to case-based surveillance for infectious diseases, including monkeypox virus (MPXV). WWS can detect pathogen circulation independent of healthcare-seeking behavior and testing availability and may provide early warning of outbreaks. We piloted WWS during an monkeypox outbreak in Kenya that has been ongoing since July 2024. Wastewater specimens were collected from two sources: 25 routine polio surveillance sites, sampled monthly from June 2024 to December 2025 and four sites established specifically for this project in high-risk communities along Kenya's Northern Transport Corridor (NTC) and near the North Star Alliance roadside clinics, sampled weekly between June 2025 and October 2025. These targeted sites were selected to capture populations at higher risk for monkeypox. Grab samples were concentrated at the Kenya Medical Research Institute (KEMRI) polio laboratory, with nucleic acid extraction and MPXV PCR performed at a CDC-supported KEMRI laboratory in Nairobi. Among 394 samples collected from routine polio surveillance sites, one (0.3%) tested positive for MPXV from Kibera, a high-density urban informal settlement in Nairobi. No positive samples were detected in Mombasa, the outbreak epicenter. Among 80 samples collected from targeted sites, six (7.5%) tested positive. MPXV detection was intermittent and did not consistently correlate with reported case counts. This pilot demonstrated the feasibility of monkeypox WWS in Kenya and its successful implementation during an outbreak in a resource-constrained setting. Targeted sampling of high-risk populations along the NTC yielded greater positivity than sampling large general population sewer sheds. WWS has potential to strengthen monkeypox surveillance by providing data on viral circulation trends and supporting more targeted public health messaging on potential community cases. However, additional work is needed to determine optimal sampling sites and to better understand how WWS data relates to case-based surveillance.

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HELMINTH AND MALARIA CO-INFECTION AMONG PREGNANT WOMEN IN BATTOR AND ADIDOME TOWNS OF THE VOLTA REGION OF GHANA

Sarah Alhakimi[1], Navneet Kaur[2], Javeriya Choudry[1], Naa Adjeley Frempong[3], Charity Ahiabor[4], William Kofi Anyan[3], Abraham K. Anang[3], **Nilanjan Lodh**[1]

[1]Department of Medical Laboratory Science, Marquette University, Milwaukee, WI, United States, [2]Department of Biomedical Sciences, Marquette University, Milwaukee, WI, United States, [3]Parasitology

Department, Noguchi Memorial Institute for Medical Research, University of Ghana, Accra, Ghana, [4]Science Laboratory Technology, Accra Technical University, Accra, Ghana

In sub-Saharan Africa, approximately 40 million pregnant women are exposed to parasitic diseases such as malaria caused by *Plasmodium falciparum*, Schistosome parasites, and soil-transmitted helminths. The co-infection can lead to consequences for the child, such as intrauterine growth retardation, low birth weight, pre-term delivery, and neonatal mortality. The objective of the study was to determine the nature and extent of coinfection from 100 samples collected from the Battor (50) and Adidome (50) districts of Ghana. Out of 50 for the Adidome district determined for *P. falciparum* by RDT, Malaria PAN, and Malaria Pf kit, 39 were true positive (TP), 8 were true negative (TN), and 30 were false negative (FN). For Battor, 19 were TP, 12 TN, and 20 FN. For *Schistosoma mansoni* in Adidome via polymerase chain reaction (PCR) and loop-mediated isothermal amplification (LAMP), 21 tested positive, and 29 were negative, with 52.5% sensitivity and 100% specificity. For *S. haematobium,* 28 were positive and 22 negative using PCR with 70% sensitivity and 100% specificity. In LAMP, 28 were positive, and 22 were negative, with 70% sensitivity and 100% specificity. In Battor PCR for *S. mansoni,* 28 positives and 22 negatives with 68.3% sensitivity and 100% specificity. In LAMP, 32 were positive, and 18 were negative, with 80% sensitivity and 100% specificity. For S*. haematobium,* PCR showed 30 positive and 20 negative, with 73.2% sensitivity and 100% specificity. With LAMP, 21 were positive, and 29 negatives, with 51% sensitivity and 100% specificity. In both district age groups, B (20-30 years) had the highest infection prevalence for *P. falciparum, S. mansoni, S. haematobium,* and *Strongyloides stercoralis*. In conclusion, the results will be utilized as a part of the continuous surveillance for future research aiming at gathering nationally representative data in Ghana on the prevalence of coinfection and proposing interventions based on that for the vulnerable pregnant women population.

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CLINICAL RESEARCH PLATFORMS TO INVESTIGATE EMERGING INFECTIOUS DISEASES IN AN EVOLVING GLOBAL HEALTH LANDSCAPE

Aaron T. Neal[1], Robert Sinto[2], Jamila Aboulhab[1], Guillermo M. Ruiz-Palacios[3], Kade Kallon[1], Abdoul H. Beavogui[4], Courtney L. Renken[1], Soka Moses[5], Esther G. Akpa[1], Sophia Siddiqui[1], **Stephen Migueles**[1]

[1]National Institute of Allergy and Infectious Diseases, Rockville, MD, United States, [2]Faculty of Medicine, Universitas Indonesia - Cipto Mangunkusumo National Hospital, Jakarta, Indonesia, [3]National Institute of Medical Sciences and Nutrition Salvador Zubirán, Mexico City, Mexico, [4]National Center for Training and Research in Rural Health, Mafèrinyah, Guinea, [5]Partnership for Research on Vaccines and Infectious Diseases in Liberia, Monrovia, Liberia

Since the early 21st century, significant outbreaks and epidemics have included H5N1 (2004-2014, 2024-2025), H1N1 (2009-2010), Ebola (2014-2016, 2018-2020), Zika (2015-2016), COVID-19 (2020-2023), and monkeypox (2022-2024). These events underscore the inevitability of future epidemics, despite persistent uncertainty regarding their timing and geographic origin. Early outbreak response efforts depend heavily on current knowledge of disease epidemiology, availability of accurate diagnostics, and access to effective medical countermeasures. Inter-epidemic periods provide critical opportunities for strengthening preparedness through clinical research to improve patient outcomes for existing endemic diseases and emerging infectious diseases. In this context, the National Institute of Allergy and Infectious Diseases (NIAID) established clinical research platforms with public health authorities in Mexico, Guinea, Liberia, Democratic Republic of the Congo, and Indonesia during epidemic events from 2009 to 2018. These platforms are multidisciplinary and disease-agnostic, supporting the conduct of collaborative clinical research while maintaining readiness to pivot rapidly in response to emerging infectious disease threats at their source. The platform model has demonstrated success: approval of the first Ebola vaccine relied on data from the research platforms in Liberia and Guinea, approval of the first Ebola monoclonal antibodies relied on data from the platforms in Liberia and DRC, and local COVID-19 diagnostic capability was led by the platforms in Indonesia, Liberia, and Guinea. Since national governments are primarily responsible for public health emergency responses, the research platform model offers distinct advantages by developing sustained and transparent research capability, enabling rapid reprioritization aligned with national needs, and maintaining direct communication between partners. Expanding research platforms can further accelerate the generation of evidence needed to mitigate the impact of future epidemics while meeting goals of enhancing research capabilities globally.

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SPEED, STRENGTH, AND STEALTH: RETHINKING THE CONTROLLABILITY OF AN INFECTIOUS DISEASE OUTBREAK

Michael E. DeWitt[1], Nicholas Kortessis[2]

[1]Wake Forest University School of Medicine, Winston Salem, NC, United States, [2]Wake Forest University, Winston Salem, NC, United States

The emergence and rapid spread of highly pathogenic avian influenza(HPAI) A(H5N1) in US dairy cows on farms poses a growing threat to the food supply and zoonosis to humans. During the outbreak beginning in March 2024, the virus spread between farms across multiple states largely unabated despite multiple factors suggesting control: intense monitoring and surveillance at farm and interstate levels; restriction of interstate movement; seeming lack of asymptomatic period, and an ability to isolate and quarantine infected cows on farms. Initial farm-level outbreaks imply a moderate basic reproduction number, suggesting some unrecognized factor allowed for uncontrolled spread of this pathogen. Classical framing of the "controllability" of an infectious disease outbreak emphasizes the basic reproduction number and the proportion of infections caused during the asymptomatic period. By these metrics, H5N1 should have been manageable. We evaluate whether another dimension of disease controllability—pathogen speed, as measured by spread within farms—is a viable explanation for early H5N1 spread. To assess the relative importance of speed, we modeled within farm spread and a variety of surveillance and control measures. For each, we examined the relative importance of time delays between the first infection and the time of intervention (and thus speed) versus asymptomatic infections on epidemic spread. We constructed measures of contribution of factors to risk: the partial relative risk of infection due to delay and separately, the partial relative risk of infection due to asymptomatic infection to compare the contribution of each. We explore these measures in several other infectious diseases of human importance to identify contexts under which different surveillance frameworks (e.g., the 7-1-7 framework) may detect an outbreak, too late for reasonable containment. We found that this is the case for H5N1. This research illustrates the capacity for epidemic speed to shape disease mitigation and policy intervention effectiveness.

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PANDEMIC PREPAREDNESS: USING BLOOD-BANKING CRITERIA TO ESTABLISH "HEALTHY CONTROL" BASELINE REFERENCE MATERIAL FOR AN INTERNATIONAL VIRTUAL BIOREPOSITORY SYSTEM

Nikaash Pasnoori[1], Zoe Steinberg[2], Shahjahan Ali[3], Amy Price[4], May C. Chu[5], Judith Giri[3]

[1]University of Colorado Anschutz School of Medicine, Aurora, CO, United States, [2]Heidelberg University, Heidelberg, Baden-Württemberg, Germany, [3]Colorado Center for Global Health, Aurora, CO, United States, [4]Dartmouth Institute for Health Policy & Clinical Practice, Lebanon, NH, United States, [5]Colorado School of Public Health, Aurora, CO, United States

The COVID-19 pandemic highlighted a preparedness gap: there was no trusted resource for rapidly sharing biospecimen samples critical for vaccine efficacy, diagnostics, and research. The Virtual Biorepository System (VBS) aims to fill the gap for access to quality specimens especially of representative samples that accommodate the diversity of global communities' exposures to disease and show the feasibility of sharing globally representative serological specimens as reference materials for

accurate baseline measurements. Reference baseline blood samples are collected from ten "healthy control" subjects at the 10 VBS low-and-middle-resource countries. Our definition of a "healthy control" (HC) is based on comparisons between blood donation regulations (BDRs) of each national site. The WHO guidelines were used as an overarching comparator. Categories were delineated within the regulations (age, weight, transfusion history, etc.). From this comparison, common BDR criteria included transfusion-transmissible infections, with the four most screened being HIV, hepatitis B, hepatitis C, and syphilis. Several chronic conditions were listed as exclusion criteria: hypertension, malignancy, and cardiovascular disease. The commonalities found within each of the 10 countries' BDRs establish their use as appropriate frameworks for creating a high-level global consensus definition of HC. When a new emerging threat of pandemic proportions arises, trusted and validated HC samples are critical to differentiate disease conditions from local community exposures. Prior to our effort, a common HC framework was not established. This assessment will help create future accessible and well-defined HC specimen collections to support pandemic countermeasure developments.

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PREPARING FOR THE NEXT PANDEMIC; HOW INCOME, NOT SYMPTOMS, SHAPED COVID-19 TESTING IN GHANA

Kenneth Bentum Otabil, Jude NA Okine, Victoria Ayanyemi
University of Energy and Natural Resources, Sunyani, Ghana

Equitable diagnostic access is central to effective pandemic response, yet inequities in testing can distort surveillance and delay control efforts. The COVID-19 pandemic provides a critical opportunity to assess whether testing uptake reflects clinical need or socioeconomic advantage. We examined how socioeconomic status (SES) influenced diagnostic access, preventive behaviors, and psychological distress in Ghana. We conducted a cross-sectional study of 1,000 respondents in Ghana (2020-2021) using questionnaires deployed on Kobo collect app on numeric tablets. Multivariate logistic regression models estimated adjusted odds ratios (AORs) for COVID-19 testing and significant psychological distress (SPD), controlling for age and symptom status. Interaction terms (SES × symptomatic status) were used to identify differential effects across income groups. Income, not symptoms, was the dominant determinant of testing. Each increase in SES was associated with higher odds of COVID-19 testing (AOR: 2.09; 95% CI: 1.61-2.72; $p < 0.001$), independent of symptomatic status, indicating systemic inequity in diagnostic access. Preventive behavior adherence was high overall (90.0%), but lower among low-income individuals (82.1%) compared to complete adherence in high-income groups. SPD prevalence was 16.7%. Higher SES independently predicted increased distress (AOR: 1.89; $p < 0.001$). However, interaction modeling revealed two distinct pathways: among low-income individuals, flu-like symptoms doubled the likelihood of distress, with food insecurity acting as a marginal mediator (AOR: 1.43; $p = 0.075$); among higher-income individuals, distress was driven primarily by social and informational disruption. COVID-19 testing in Ghana systematically favored wealth over clinical need, undermining equitable surveillance. Divergent socioeconomic pathways of distress further expose the limitations of uniform public health strategies. Pandemic preparedness must prioritize decentralized, cost-free diagnostics and targeted mental health interventions tailored to socioeconomic context.

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DENGUE AND CHIKUNGUNYA SEROPREVALENCE FROM 2012-2022 IN SOUTHERN MALAWI

Abra Rachida Koudjra[1], Sudhaunshu Joshi[1], Amanuel Wolde[1], Gabriella Meshesha[1], Megan Kowalcyk[1], Nginache Victoria Nampota-Nkomba[1], Lauryn Butler[1], JeanPaul Courneya[1], Felix Mkandawire[2], Osward Mulyawaka Nyirenda[2], Rhoda Masonga[2], A. Desiree LaBeaud[3], Matthew B. Laurens[1], Miriam K. Laufer[1], Andrea G. Buchwald[4]
[1]Center for Vaccine Development and Global Health, University of Maryland School of Medicine, Baltimore, MD, United States, [2]Blantyre Malaria Project, Blantyre, Malawi, [3]Stanford University, Stanford, CA, United States, [4]Department of Clinical Sciences,Liverpool School of Tropical Medicine, Liverpool, United Kingdom

In 2023, the WHO reported 29 arbovirus outbreaks in Africa with 19,569 confirmed cases, most due to dengue. Arbovirus outbreaks have been linked to climate change with evidence of changing distributions globally. While evidence of arbovirus circulation exists in numerous sub-Saharan African (SSA) countries, a lack of surveillance and testing restrict understanding of their spatiotemporal distribution. In southern Malawi, cross-sectional seroprevalence studies have found evidence of arbovirus circulation, however incidence rates are unknown. As SSA undergoes rapid urbanization and climate shifts, it is crucial to assess the distribution and changes in arbovirus seroprevalence over the past decade. We investigated the seroprevalence and incidence of dengue (DENV) and chikungunya (CHIKV) from three longitudinal studies in southern Malawi. Study 1 (2012-2014) enrolled pregnant women without HIV at 20-26 weeks gestational age randomized to either sulfadoxine-pyrimethamine or chloroquine (CQ) prophylaxis during pregnancy. Study 2 (2015-2018) followed HIV positive adults randomized to either daily trimethoprim-sulfamethoxazole TS, weekly CQ, or no prophylaxis. Study 3 (2018-2022) enrolled pregnant women between 20-36 weeks gestation into two cohorts based on HIV status. One to three samples per individual were tested for anti-DENV IgG and anti-CHIKV IgG. Annual seroprevalence was defined as the proportion of participants ever IgG-positive in that year. Of 5247 samples, (Study 1: n = 691; Study 2: n =2663; Study 3: n =1893), 120 (2.3%) were DENV-IgG positive. Among 1967 completed CHIKV assays, 376 (19%) were CHIKV-IgG positive. Preliminary results show DENV seroprevalence remained stable over time (2.9% in 2012-2014; 3.4% in 2015-2018; 3.3% in 2018-2022). CHIKV seroprevalence declined significantly over time from 34% in 2018 to 18% in 2022 ($p <0.0001$). Testing for CHIKV from 2012 to 2018 is ongoing. Final analysis will identify incident infections via seroconversion. Regression models will assess seropositivity by year and evaluate clinical, climatic, and demographic predictors of arbovirus incidence and prevalence.

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ECOLOGICAL STRUCTURING OF TICK VIROMES ACROSS EAST AFRICA REVEALS MICROBIAL INTERACTIONS THAT MAY INFLUENCE ARBOVIRUS TRANSMISSION

Victor O. Anyango[1], LK Kiio[1], BA Kwasi[1], M. Lukindu[2], T. Nakayaki[2], J. Lutwama[2], L. Ward-Polonski[1], JK Kayondo[2], J. Mutisya[3], FM Musili[3], J. Lutomiah[3], CL Brelsfoard[1], MG Onyango[1]
[1]Texas Tech University, Lubbock, TX, United States, [2]Uganda Virus Research Institute, Entebbe, Uganda, [3]Kenya Medical Research Institute, Nairobi, Kenya

Ticks harbor complex microbial communities that include viruses, bacteria, and symbiotic microorganisms. While the diversity of tick-borne pathogens is well documented, a significant knowledge gap remains regarding the structural organization and intermicrobial interactions within these complex communities. Understanding these ecological dynamics is particularly important in East Africa, a region that represents the biological frontier, offering a high-contrast landscape of host-pathogen diversity in a historically under-sampled, yet critical, viral hotspot. In this study we hypothesized that the tick virome will exhibit a non-random structure correlated to specific ecological determinants, including host species specificity, geographic distribution, and the composition of the internal bacterial microbiome. Using high-throughput metagenomic sequencing, we characterized the eukaryotic virome and bacterial microbiome of ticks collected from pastoralist ecosystems in Kenya and Uganda. We observed a strong ecological structuring by tick genus and geographic location. *Hyalomma* spp. ticks collected in northern Kenya formed a tight cluster with highly similar viral communities, whereas *Rhipicephalus* spp. ticks from Uganda displayed greater dispersion, indicating increased virome heterogeneity across sampling sites. Comparative analysis of viral and bacterial communities revealed that ticks with richer bacterial microbiomes tended to harbor more diverse viromes. Network analyses further identified clusters of viruses and bacteria that consistently co-occurred across samples, suggesting

potential ecological interactions within the tick holobiont. These findings suggest that tick-associated viromes are not randomly assembled but are shaped by host identity, geographic environment, and microbial context. Understanding these microbial ecosystem dynamics may provide new insights into factors influencing arbovirus persistence and transmission risk in endemic regions

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ENTOMOLOGICAL INVESTIGATION REVEALS CIRCULATION OF CRIMEAN CONGO HEMORRHAGIC FEVER VIRUS (CCHFV) IN NAIROBI COUNTY, KENYA

Sancto Yalwala Jumba[1], David Ochieng Oullo[1], Nicholas Okoth Odemba[1], Francis Ngere[1], Charles Waga[1], Richard Ochieng Odinga[1], Jane Thiiru[1], Gladys Kerich[2], Lucy Isabella[1], Janet Ambale[1], Jacob Mwangi[1], Elizabeth Kioko[1], Joel Lutomiah[3], John Eads[4]

[1]Walter Reed Army Institute of Research-Africa, Kisumu, Kenya, [2]Walter Reed Army Institute of Research-Africa, Kismu, Kenya, [3]Kenya Medical Research Institute (KEMRI), Nairobi, Kenya, [4]Walter Reed Army Institute of Research-Africa, DPO, AE, United States

Nairobi is the only capital city in the world with a national park within its borders. The presence of livestock in parts of Nairobi during times of drought results in extensive human-livestock-wildlife interaction. Despite this, no entomologic investigation targeting ticks has been conducted in the city to determine the diversity of tick species and the risk of transmission of tick-borne pathogens. During the Justified Accord 2026 military exercise, we conducted vector surveillance in Nairobi, Isiolo, Laikipia and Samburu Counties to assess the risk of human exposure to tick-borne pathogens. Between 24.02.2026 and 08.03.2026, we sampled ticks from livestock at the Humanitarian Peace Support School (HPSS) in Nairobi and the surrounding environments by animal grooming and dragging methods. We also sampled at the Counter Insurgency Terrorism and Stability Operations (CITSO) in Laikipia County, School of Infantry (SOI) in Isiolo County and School of Armory (SOA) in Samburu County. The ticks were morphologically identified to species, pooled and screened for tick-borne pathogens using RT-PCR. N=1,285 (HPSS, n=833, SOI, n=286, SOA, n=85 and CITSO, n=81) ticks were collected, identified to 11 species and pooled (n=244). *Rhipicephalus microplus* comprised 21% (n=268), *Rh. pravus* (18%, n=233), *Haemaphysalis leachi* (15%, n=191), *Rh. evertsi evertsi* (15%, n=187), *Rh. appendiculatus* (13%, n=164), *Hyalomma marginatum rufipes* (7%, n=86), *Rh. sanguineus* (5%, n=59), *Amblyomma gemma* (4%, n=53) and others (<3%). One pool of *Rh. evertsi evertsi* that comprised 8 male ticks collected from sheep in Nairobi tested positive for CCHF virus. These results mark the first detection of CCHFV in Nairobi County. Detection in *Rh. evertsi evertsi* further confirms other tick species as vectors of CCHF. The convergence of wildlife, livestock and humans in parts of Nairobi County including the HPSS and Langata Barracks which neighbors Nairobi National Park makes these areas suitable habitats for transmission of CCHFV and other zoonotic pathogens. This calls for continued surveillance for early detection, understanding disease patterns and inform control policies.

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COMPARISON OF INSECT MIGRATION AT ALTITUDE VS. GROUND ACTIVITY: IMPLICATION FOR PUBLIC HEALTH AND FOOD SECURITY IN GHANA

Rita Nartey Frimpong[1], Lourdes Chamorro[2], Matthew Buffington[2], Yaw A. Afrane[3], Abdul R. Mohammed[3], Christopher Mfum Owusu-Asenso[3], Adama Dao[4], Alpha S. Yaro[4], Roland Bamou[5], Kwasi Obiri-Danso[1], Tovi Lehmann[5]

[1]Kwame Nkrumah University of SCience and Technology, Kumasi, Ghana, [2]Smithsonian Institution, National Museum of Natural History, Washington DC, DC, United States, [3]University of Ghana, Accra, Ghana, [4]Malaria Research and Training Center (MRTC)/Faculty of Medicine, Bamako, Mali, [5]National Institutes of Health, Rockville, MD, United States

Work on windborne insects in Africa emphasized a few crop pests such as locusts and the African armyworm moths. Recent studies have revealed multitude of insects migrating at altitude (40-290 m above ground), exposing gaps in our knowledge of these migrants' identity, services they provide, sources and destinations, flight range, schedules, and drivers among other topics. Filling these gaps would help address Africa's growing nutritional needs and public health problems. Here, in the first study of windborne insects over Ghana, we describe the aerial fauna and key patterns of its movement >100 m above ground. Sticky nets (panels) mounted on tethered helium balloons between 160 and 190 m above the ground, were used to sample high-altitude flying insects in a year-long survey. Concurrent samples collected from a sticky net mounted 1 m above ground were used to represent insects flying near the ground. Insects collected over multiple months from two sites in the equatorial forest were examined. Specimens were identified morphologically to order and to morphospecies, counted, and recorded. Results show that windborne insect migration occurs regularly at altitude with mild seasonal variation over equatorial forests. Yet, rare mass-migration events occur in certain taxa. Overall, ground density predicts aerial density, accounting for 40% of the variance in aerial density, suggesting a "constant" fraction of the insect fauna engages in high-altitude flight. Among the high-altitude migrants are important pests that probably affect public health and food security. Overall, the same insect orders that dominate near the ground also dominate the aerial fauna, but at altitude Diptera (flies), Coleoptera (beetles) and Homoptera (planthoppers) predominate; groups that have high share of notorious pests. This study opens new opportunities for understanding the sources and destinations of many species impacting human, animal, and plant wellbeing.

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CHALLENGES IN SAND FLY SALIVARY GLAND COLLECTION AND DISSECTION

Ashleigh Hale[1], Ngwa Niba Rawlings[2], Steven Montgomery-Laird[1], Orin Courtenay[3]

[1]University Hospital Coventry, Coventry, United Kingdom, [2]School of Life Sciences, University of Warwick, Warwick, United Kingdom, [3]University of Warwick, Warwick, United Kingdom

Leishmaniasis is a parasitic disease caused by protozoa of the genus *Leishmania*, transmitted through the bites of infected female phlebotomine sand flies. During blood feeding, female sand flies inject salivary proteins and, if present, infectious promastigotes, into the host's skin. A significant number of cutaneous leishmaniasis cases have been reported from the British military personnel undertaking jungle warfare training (JWT) in Belize. Current epidemiological studies investigating exposure to sand flies includes detecting human antibodies to sand fly salivary proteins injected during blood feeding. The process of obtaining the salivary antigens requires careful dissection of the salivary glands of freshly killed sand flies. This study evaluated the feasibility of field collection, dissection, and storage of sand fly saliva glands to support the development of a serological assay for assessing exposure risk in military personnel. CDC light traps were positioned 30 metres apart, in an alternating placement pattern, approximately 30cm above the ground. This arrangement allowed sampling of sand flies around the entirety of the troops' temporary camp areas. Traps were operated continuously for 12 hours, from 6pm to 6am, from 10th - 28th November 2025. Following collection, the captured sand flies were euthanised using 70% alcohol spray and stored in phosphate buffer saline (PBS). Dissection involved immobilising the sand fly on a slide, detaching the head, and pulling the glands from the thorax using microdissection needles under a stereomicroscope. Dissection was time consuming and labour intensive, requiring specialist equipment and trained individuals for successful salivary gland collection. Fast transport was required from jungle to laboratory to maintain the structure of the sand flies. In addition, a cold chain (with generator backup) was required to transport glands back to the UK. The detailed process highlighted the difficulty in obtaining sand fly saliva for epidemiological studies, and the need for improved methodologies to truly understand the epidemiology of sand flies and the diseases they transmit.

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ECOLOGICALLY STRUCTURED GENETIC DIVERSITY IN *SIMULIUM DAMNOSUM* ACROSS GHANA: CONSEQUENCES FOR ONCHOCERCIASIS ELIMINATION

Millicent Opoku[1], Neha Sirwani[2], Kwadwo K. Frempong[3], Sampson Otoo[3], Seduo Naniogou[4], Philomena Jackson[3], Millicent S. Afatodzie[3], Sellase Akosua Pi-Bansa[3], Joseph Harold Nyarko Osei[5], Franklin Ayisi[3], Abena A. Nyarko[3], Abena A. Nyarko[3], Sarah M. Dogbe[3], Warwick N. Grant[6], Daniel Boakye[7], Shannon Hedtke[8]

[1]La Trobe Institute of Molecular Sciences/Department of Microbiology, Anatomy, Physiology and Pharmacology, La Trobe University/NMIMR, Melbourne, Australia, [2]Department of Microbiology, Anatomy, Physiology and Pharmacology, La Trobe University, Melbourne, Australia, [3]Department of Parasitology, Noguchi Memorial Institute for Medical Research (NMIMR), College of Health Sciences, University of Ghana, Accra, Ghana, [4]The END Fund, New York, NY, United States, [5]Biomedical and Public Health Research Unit, Water and Research Institute, Council for Scientific and Industrial Research (CSIR), Accra, Ghana, [6]2.Department of Microbiology, Anatomy, Physiology and Pharmacology, La Trobe University, Melbourne, Australia, [7]The END Fund/Noguchi Memorial Institute for Medical Research, New York, NY, United States, [8]La Trobe Institute of Molecular Sciences/ Department of Microbiology, Anatomy, Physiology and Pharmacology, La Trobe University, Melbourne, Australia

Onchocerciasis remains a significant public health burden in Ghana despite decades of ivermectin-based control under the African Programme for Onchocerciasis Control (APOC) and its successor programmes. The effectiveness of elimination efforts depends critically on understanding the population biology of *Simulium damnosum*, the blackfly vector, yet genetic data from Ghana remain limited. This study characterised the population genetics structure of *S. damnosum* across 24 localities in Ghana. Genomic DNA was extracted from 632 *S. damnosum* specimens and submitted for short-read whole genome sequencing. Using complementary methods (DAPC and ADMIXTURE) we identified five genetically distinct population clusters aligned with Ghana's Guinea Savannah, Sudan Savannah, Transitional Zone, and Semi-Deciduous Forest (SDF) agro-ecological zones (ecozones). Critically, the SDF zone harboured two deeply divergent genetic lineages: a western cluster centred on Bosomase (SDF-W) and an eastern cluster comprising Dodo Tamale, Elavanyo and Holuta (SDF-E). This finding challenges the assumption of homogeneous vector populations within shared ecological zones and has direct relevance for control strategies. Populations at ecozone boundaries displayed elevated admixture, identifying potential corridors of gene flow that may facilitate the spread of parasite strains between zones. The strong concordance between genetic clusters and ecological boundaries suggests that vector control strategies such as aerial larviciding, and surveillance should be designed at the level of genetically defined units rather than administrative or ecological boundaries alone. These data provide a critical genomic baseline for evidence-based onchocerciasis elimination planning in Ghana.

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GENOME OF *WOLBACHIA* ENDOSYMBIONT ASSOCIATED WITH SAND FLY VECTOR OF LEISHMANIASIS

Alexandra Mushegian[1], Matthew Chung[1], Carolina Catta-Preta[1], Eva Iniguez[2], Claudio Meneses[3], Pedro Amado Cecilio[4], David L. Sacks[5], Shaden Kamhawi[6], Elodie Ghedin[7]

[1]NIAID/NIH, Bethesda, MD, United States, [2]NIH/NIAID, Rockville, MD, United States, [3]Fiocruz - Oswaldo Cruz Foundation, Rio De Janeiro, Brazil, [4]LMVR, NIAID, NIH, Rockville, MD, United States, [5]National Institutes of Health, NIAID/Lab of Parasitic Diseases, Bethesda, MD, United States, [6]Natl Institutes of Health, Cambridge, United Kingdom, [7]Institute for Genomic Research, Rockville, MD, United States

Sand flies (family Phlebotominae) are blood-feeding insects that transmit several species of the parasitic protozoan *Leishmania,* which causes cutaneous and visceral forms of leishmaniasis. *Leishmania* transmission and infection intensity are influenced by the sandfly-associated microbial community, but little is known about how the variation in the composition of the microbiome contributes to variation in sand fly fitness and vector competence in the wild or in the laboratory. We characterized the microbiomes of larvae and adults of multiple generations of sand flies from laboratory colonies. Initial community profiling of the bacterial microbiota revealed that a particular colony of *Phlebotomus papatasi*, which historically experienced a reduction in its capacity to transmit experimental infections, contained a sub-population of individuals infected with the arthropod endosymbiont *Wolbachia.* When exposed to *Leishmania major* via artificial blood-feeding, *Wolbachia*-positive females exhibited a lower total parasite burden and fewer infectious metacyclics than *Wolbachia*-negative individuals in this colony, suggesting that a rise in *Wolbachia* prevalence may have been responsible for reduced vector competence. *Wolbachia's* effects on insect reproduction and refractoriness to infection have been extensively studied in mosquito vectors, but have barely begun to be investigated in sand flies. We are sequencing and assembling the genome of *P. papatasi*-associated *Wolbachia* in order to begin to assess the endosymbiont's role in this vector's biology and potential as part of the vector control toolkit.

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ARBOVIRUS SCREENING AND ASSESSMENT OF COMMUNITY KNOWLEDGE-LEVEL, SOCIOENVIRONMENTAL AND BEHAVIOURAL DRIVERS OF MOSQUITO-BORNE ARBOVIRUS TRANSMISSION IN GHANA

Obadiah Opoku-Bamfoh, Abdul-Hakim Mutala, Augustina Sylverken, Kingsley Badu

Kwame Nkrumah University of Science and Technology, Department of Theoretical and Applied Biology, Kumasi, Ghana

Mosquito-borne arboviruses are an emerging public health concern in West Africa, but their prevalence and community-level transmission drivers remain poorly understood. This study integrates arbovirus screening in mosquito populations with assessment of community knowledge levels of mosquito-borne arboviruses and determines socioenvironmental factors and behavioural risk drivers of mosquito-borne arbovirus transmission in Ghana. Mosquitoes were collected from five selected communities in Western North and Ahafo Region, using Biogent Sentinel 2 traps, CDC light traps, and Human Landing Catches (HLC). Mosquitoes were identified morphologically using a stereomicroscope, pooled and screened for Dengue virus (DENV), Yellow fever virus (YFV), Zika virus (ZIKV), and Chikungunya virus (CHIKV) using real-time PCR. A structured questionnaire was administered to assess knowledge, socioenvironmental and behavioural risk drivers of arbovirus transmission. Overall, 6,799 mosquitoes were collected, largely *Culex species* (73.18%), followed by *Anopheles* (23.25%), *Mansoni* (2.37%) and *Aedes* (1.19%) populations. A total of 45 pools, comprising 815 female mosquitoes, were screened, and no arboviral RNA was detected. However, the questionnaire findings revealed widespread exposure risk (96.6%), with many participants engaging in activities that increase the risk of arbovirus transmission. Knowledge on arboviruses and breeding sites of mosquitoes was significantly low (p=0.001). Residents in rural communities were at significantly higher risk of exposure compared with urban and peri-urban populations (p=0.001). Although no arboviruses were detected in mosquitoes collected at the time of sampling, the presence of substantial socioenvironmental and behavioural risk factors indicates vulnerability to arbovirus transmission. We highlight the importance of an integrated entomological and community-centred surveillance approach to inform preparedness for arbovirus outbreaks and modifiable environmental and behavioural risk factors associated with arbovirus transmission.

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GENETIC DIVERSITY AND EVIDENCE OF REASSORTMENT OF CRIMEAN CONGO HEMORRHAGIC FEVER VIRUS IN GHANA

Seth Offei Addo[1], Eric Behene[1], Selassie Kumordjie[1], Richard Odoi-Teye Malm[2], Patrick Kwasi Obuam[3], Sandra Abankwa Kwarteng[3], Ellis Owusu-Dabo[3], Anne T. Fox[4], Andrew G. Letizia[1], James Harwood[5], Shirley Nimo-Paintsil[1], Samuel Kweku Dadzie[1]

[1]Noguchi Memorial Institute for Medical Research, Accra, Ghana, [2]Noguchi Memorial Institute for Medical Research, Kumasi, Ghana, [3]Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, [4]U.S. Naval Medical Research Unit EURAFCENT, Ghana Detachment, Accra, Ghana, [5]U.S. Naval Medical Research Unit EURAFCENT, Sigonella, Italy., Sigonella, Italy

Crimean-Congo hemorrhagic fever virus (CCHFV) is a tick-borne zoonotic virus with a broad geographic distribution and high genetic variability, driven in part by genome segment reassortment. Although transmission has increased across Africa, epidemiological data on viral diversity and reassortment remain scarce. This study investigated the genetic diversity and evolutionary dynamics of CCHFV in ticks sampled from slaughtered cattle at the Kumasi abattoir. The ticks were morphologically identified, screened for the virus using RT-qPCR targeting the S segment of the CCHFV genome, and further subjected to whole-genome sequencing. Of the 768 ticks collected in 2020, *Amblyomma variegatum* (65%) was the predominant species. The prevalence of CCHFV was 0.26% in *Hyalomma truncatum* (0.13%) and *H. rufipes* (0.13%). A strain was successfully recovered from *H. rufipes* and compared with previously identified strains in Ghana. Phylogenetic analysis showed that the S and L segments clustered within Genotype III (Africa 3), consistent with previously reported strains from Ghana and West Africa. However, the M segment clustered within Genotype I (Africa 1), associating closely with a strain previously found at the Navrongo abattoir. Likelihood-based topology analyses indicated significant divergence between the M segment phylogeny and those of the S and L segments, providing strong support for genome segment reassortment. Haplotype network analyses also revealed distinct mutational lineages among Ghanaian strains, indicating either ongoing local diversification or multiple viral introductions. These findings show that CCHFV evolution in Ghana is actively shaped by reassortment. The detection of reassorted strains in ticks collected at abattoirs underscores the importance of these settings as key interfaces where viral interactions and zoonotic spillover may occur. Strengthening whole-genome surveillance within a One Health framework would support ongoing monitoring of CCHFV genetic diversity and facilitate the timely identification of emerging public health concerns in West Africa.

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VECTOR DISTRIBUTION AND INSECTICIDE RESISTANCE STATUS OF *AEDES* MOSQUITOES IN SOUTHWESTERN GHANA

Faustina Adobea Owusu

University of Ghana, Accra, Ghana

Arboviral diseases are an emerging and under-recognized public health threat in Ghana, driven by the widespread presence of *Aedes* mosquito vectors and increasing environmental suitability for transmission. The recent detection of dengue in 2024 highlights the country's vulnerability to arboviral outbreaks, yet critical gaps remain in our understanding of vector distribution and insecticide resistance patterns, particularly in southwestern Ghana. Addressing these gaps is essential for designing effective, evidence-based vector control strategies. This study therefore assessed the distribution and insecticide resistance status of *Aedes* vectors across selected communities in southwestern Ghana. Adult *Aedes* mosquitoes were sampled using BG-Sentinel traps, Human Landing Catches and Prokopack aspirators, while *Aedes* larvae were collected from outdoor water-holding containers. WHO susceptibility assays and PBO synergist tests were used to assess phenotypic resistance, and allele-specific PCR was done to detect kdr mutations (V1016I, F1534C, V410L) in *Aedes* vectors. *Aedes aegypti* were the most abundant species (87.8%), followed by the *Ae. albopictus* (12.2%). The most predominant breeding habitat for *Aedes* mosquitoes were car tyres (66.4%). The HLC sampling technique (88.1%) was the most effective method for sampling adult *Aedes* mosquitoes. Resistance to deltamethrin (75%) and permethrin (74.3%) was observed, with full susceptibility to pirimiphos-methyl (100%) in *Aedes aegypti*, while *Aedes albopictus* mosquitoes were fully susceptible to deltamethrin (98.8%), permethrin (100%) and pirimiphos-methyl (100%). Kdr mutations were detected in both species; *Ae. albopictus* had lower frequencies of F1534C (0.18), V1016I (0.00), V410L (0.02) whilst *Ae. aegypti* showed high allelic frequencies of F1534C (0.72), V1016I (0.50) and V410L (0.06). These findings necessitate the urgent need for sustained vector surveillance and resistance monitoring to support effective control of *Aedes*-borne diseases in Ghana.

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FROM LEG TO PROFILE: PROOF-OF-CONCEPT STUDY USING TIME-OF-FLIGHT MASS SPECTROMETRY (MALDI-TOF MS) FOR THE DETECTION OF CHAGAS DISEASE VECTORS

Jessica Rocca[1], Mailén Di Palma[2], Facundo N. Urteaga[3], Patricia Alejandra Lobbia[4], Ana L. Carbajal De La Fuente[5], Pablo Schierloh[6], Barbara Rearte[7]

[1]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET); Instituto Nacional de Enfermedades Infecciosas - ANLIS Malbrán, Ciudad Autónoma de Buenos Aires, Argentina, [2]Instituto Nacional de Enfermedades Infecciosas - ANLIS Malbrán, Ciudad Autónoma de Buenos Aires, Argentina, [3]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET); 3Instituto de Investigación y Desarrollo en Bioingeniería y Bioinformática (IIB)–CONICET UNER, Oro Verde - Entre Ríos, Argentina, [4]Unidad Operativa de Vectores y Ambiente (UnOVE)–ANLIS Malbrán, Santa María de Punilla, Córdoba, Argentina, [5]5Centro Nacional de Diagnóstico e Investigación en Endemoepidemias (CeNDIE)-ANLIS Malbrán, Ciudad Autónoma de Buenos Aires, Argentina, [6]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET); Instituto de Investigación y Desarrollo en Bioingeniería y Bioinformática (IIB)–CONICET UNER, Oro Verde - Entre Ríos, Argentina, [7]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET); Instituto Nacional de Enfermedades Infecciosas–ANLIS Malbrán, Ciudad Autónoma de Buenos Aires, Argentina

Chagas disease remains a major public health concern in Argentina, with Triatoma infestans as the primary vector. Conventional detection methods (microscopy, PCR/qPCR) present limitations, including low sensitivity and high infrastructure requirements. This study evaluates Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) as a rapid alternative for distinguishing infected and uninfected vectors. Adult T. infestans from Argentine colonies were experimentally infected with *Trypanosoma cruzi* (Tulahuen strain, DTU VI) by feeding on infected mouse blood (1×10^6 parasites/ml), while controls received uninfected blood. Infection was confirmed by microscopy 15-20 days post-infection. The femur of the left middle leg was analyzed by MALDI-TOF MS using α-cyano-4-hydroxycinnamic acid matrix. Four biological and three technical replicates produced 12 spectra per individual (28 specimens; 336 spectra total). Principal Component Analysis (PCA) and unsupervised hierarchical clustering were used for pattern recognition. PCA showed clear separation between infected and uninfected specimens, explaining 91% of total variance (PC1: 79.9%, PC2: 11.1%). Binary Discriminant Analysis identified 5 key peaks (m/z: 3467, 8500, 8524, 8560, 8564). Unsupervised hierarchical clustering showed complete separation between groups, forming non-overlapping clusters. Infected specimens showed enhanced peptide signals in the 2000-4000 m/z range, while uninfected specimens exhibited distinct profiles in the 6000-9000 m/z range. These findings show the strong potential of MALDI-TOF MS for identifying infected vectors, providing a robust proof-of-concept for rapid field diagnostics. The technique offers key advantages over conventional methods: faster analysis, minimal sample preparation, and scalability for surveillance programs. This approach could improve surveillance and vector control

strategies in endemic regions and may be extended to other medically relevant vectors, particularly in resource-limited settings where rapid, field-deployable diagnostics are needed.

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DEVELOPING *IXODES SCAPULARIS* TICK RISK MAPS AND PREDICTIVE RISK MODELS FOR NEW YORK STATE PARKS

Jillian Rose Fuss, Alexander Keyel, Melissa Prusinski
Wadsworth Center, Albany, NY, United States

Tickborne illnesses make up roughly 90% of reported vector-borne disease cases within the United States. Many of these diseases are transmitted by blacklegged ticks (*Ixodes scapularis*), which are increasing in abundance and expanding in geographic range, leading to corresponding increases in tickborne disease risk. Characterizing tick encounter risk could inform public health responses and improve public health outcomes. First, host-seeking nymphal tick density was compared between New York State (NYS) parks and non-park sampling locations, to test whether parks would be representative of surrounding areas. Second, tick density was compared to park visitation rates, to identify whether particular high-use sites also had high tick encounter risk. Finally, GIS-based risk maps were developed for selected NYS parks based on previous studies of tick encounter risk. Statistical analyses were conducted in R. It was found that nymphal tick density in NYS parks was not representative of surrounding areas, as there is regional variation in nymph density within the state, and NYS parks had higher nymph densities when compared to non-park sites. Additionally, high attendance sites did not correspond with high nymph densities for NYS parks. A fine-scale risk model was developed based on variables identified in prior research, such as elevation, vapor pressure deficit from two years prior, distance to nearest hydrographical features, and deer harvest data. The risk model will serve as a field-testable hypothesis for tick encounter risk within and across sites. The risk maps generated by the model will be used to strengthen effective risk communication to the public with the aim of preventing tick encounters in NYS parks and reducing tick-borne diseases regionally.

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VALIDATION OF A NEW HIGHLY SENSITIVE REAL-TIME PCR TARGETING CYTOCHROME C OXIDASE SUBUNIT 1 (*COX1*) IN *BABESIA MICROTI*

Brooke Clemons, Susan Madison-Antenucci
Wadsworth Center, NYSDOH, Albany, NY, United States

Primarily considered an arthropod-borne disease, babesiosis is an infection of the red blood cells in mammals caused by protozoan parasites in the genus *Babesia*. In New York State, and most of the United States, human infection is caused by *B. microti.* At the New York State (NYS) Department of Health Wadsworth Center, routine laboratory testing for babesiosis cases includes microscopy and real-time PCR detecting the 18S rDNA gene, which is found at one copy per haploid organism. Due to an increased number of low parasitemia or negative specimens received by the laboratory, a more sensitive real-time PCR was designed targeting the cytochrome c oxidase subunit 1 (COX1). COX1 sequences are highly conserved within a species and there are an estimated 20 copies per *B. microti* organism. In this study, a clinical validation was performed which evaluated the specificity, sensitivity, reproducibility and accuracy of the new assay. Two automated DNA extraction platforms, the QIAGEN QIAcube Connect and Promega Maxwell RSC 48, were assessed and DNA was amplified using the QuantStudio 7 Flex real-time PCR system. The assay was also refined for compatibility and validated on the BD Max System. The assay was found to be specific when tested against *Babesia* spp. and other tickborne pathogens. Depending on the extraction method used, the limit of detection was approximately seven to 70 copies per reaction which equates to less than 2.5 *B. microti* parasites. The results generated remained consistent when evaluating inter and intra assay reproducibility. When assessing accuracy, a 92% agreement was found when comparing results to the 18S rDNA real-time PCR assay. With an increase in sensitivity, additional positive specimens were identified which were previously negative due to low density parasitemia. The geographical range for babesiosis cases has quickly expanded in NYS, with numbers generally trending upward each year. This further highlights the importance of validating and implementing a new, highly sensitive *B. microti* COX1 real-time PCR assay for patients in NYS with potential tickborne illness.

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CANDIDATUS RICKETTSIA NICOYANA: A NEW *RICKETTSIA* SPECIES?

Paula Alfaro-Segura[1], César Rodríguez[2], Eugenia Corrales-Aguilar[1], Adriana Troyo[3], Tatiana Murillo Corrales[1]
[1]Medical Virology, Faculty of Microbiology and Centro de Investigación en Enfermedades Tropicales CIET, University of Costa Rica, San Pedro, San Jose, Costa Rica, [2]Laboratorio de Investigación en Bacteriología Anaerobia and Centro de Investigación en Enfermedades Tropicales CIET, Universidad de Costa Rica, San Pedro, San Jose, Costa Rica, [3]Laboratorio de Investigación en Vectores and Centro de Investigación en Enfermedades Tropicales CIET, Universidad de Costa Rica, San Pedro, San Jose, Costa Rica

The genus *Rickettsia* comprises a group of intracellular, gram-negative bacteria that are associated with arthropod vectors. In 2017, two isolates of *Rickettsia* sp. were obtained from a bat tick in Costa Rica. The findings of this study suggested this was a new *Rickettsia* species belonging to the spotted fever group (SFG) and phylogenetically related to *R. rickettsii*, agent of Rocky Mountain Spotted Fever. The proposed new species was named *Candidatus* Rickettsia nicoyana. To determine if *Ca*. R. nicoyana is a new species of *Rickettsia*, we performed whole-genome sequencing and comparative genomics against *Rickettsia rickettsii* and other related species. After assembly and annotation using Unicycler and Prokka, GGDC and OrthoANI for genome similarity, eggnog-mapper and KEGG pathway analysis for functional comparative genomics, and Roary for pangenome analysis. 16S rRNA phylogenetic trees indicated that the closest relatives to *Ca*. R. nicoyana are *R. rickettsii*, *R. philipii,* and *R. peacockii.* Genomic similarity metrics confirmed *Ca.* R. nicoyana as a new species: digital DNA-DNA hybridization (dDDH) values were below 92.3% (87.5%-88.1%) and the average nucleotide identity by orthology (OrthoANI) was below 99.19% (98.74%-99.11%), when compared to the closest relatives. Pangenome analysis revealed that the study strains contain 285 unique genes involved in conjugation, recombination, host interaction, and metabolic adaptation, distributed across multiple genomic regions, suggesting lineage-specific genomic diversification. Virulence factor comparison determined that the *pat2* gene is present in *Ca*. R. nicoyana, unlike *R. rickettsii*. Moreover, KEGG analyses revealed different pathways present in *Ca.* R. nicoyana but absent in *R. rickettsii*. Our results confirm that *Ca*. R. nicoyana is a new species of *Rickettsia* with differences in metabolic pathways, virulence factors, and higher gene content. From a One Health perspective, our study highlights the relevance of microbial surveillance of wildlife ectoparasites, as they pose a risk of human pathogen emergence due to increasing interactions among humans, domestic and wild animals.

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TICK AND TICK-BORNE PATHOGEN SURVEILLANCE FROM TICKS COLLECTED ACROSS ISRAEL IN 2025

Gocha Golubiani[1], Tamar Chunashvili[1], Nir Rudoler[2], Elad Eliahoo[2], Giorgi Kirkitadze[1], Medea Gejadze[1], Anano Shubashishvili[1], Shota Japarashvili[1], David Denlinger[1], Thomas Musich[1]
[1]Walter Reed Army Institute of Research Europe-Middle East, Tbilisi, Georgia, [2]Kimron Veterinary Institute, Tel Aviv, Israel

Ticks are persistent, prolific vectors that spread diverse disease pathogens that threaten the health of humans, livestock, and other animals. Therefore, surveillance programs of ticks and tick-borne pathogens have medical and agricultural value. The Walter Reed Army Institute of Research Europe-Middle East and Kimron Veterinary Institute have a shared interest in Servicemember Force Health Protection and Israel's health and agriculture preparedness. 449 ticks in 250 pools (241/250 pools of adults & 9/250

pools of nymphs) comprised of the genera *Haemaphysalis, Hyalomma,* & *Rhipicephalus* were collected off 10 different species of mammalian hosts and at 29 distinct locations across Israel. All ticks were morphologically speciated using dichotomous keys, and 14 ticks underwent molecular speciation via barcoding (CO1 gene). For pathogen detection, ticks were macerated in DNA/RNA Shield to preserve nucleic acid, and total DNA and RNA were extracted for pathogen detection. All ticks underwent pathogen testing. Genus-level qPCR and RT-qPCR were performed for different pathogens: Crimean-Congo Hemorrhagic Fever virus (CCHFV), Tick-borne Encephalitis virus (TBEV), *Anaplasma*, *Borrelia*, *Ehrlichia*, and *Rickettsia*. CCHFV and TBEV were not detected in any ticks, but bacterial genera were detected to varying degrees: 34/250 pools of ticks tested positive for *Anaplasma* (all three tick genera; collected from boars, cows, and jackals), 17/250 pools tested positive for *Borrelia* (only from *Hyalomma* and *Rhipicephalus* ticks collected from cows), 74/250 pools tested positive for *Ehrlichia* (all three tick genera; collected from boars, cows, dogs, and jackals), and 163/250 pools of ticks tested positive for *Rickettsia* (all three tick genera; collected from boars, camels, cows, dogs, horses, and jackals). Future work will include next-generation agnostic sequencing paired with RNA enrichment to verify CCFHV-negative findings and to speciate pathogens using Sanger sequencing. These data and findings warrant expanding tick collections across Israel to continue surveilling for endemic and emerging pathogens across Israel and the Levant.

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MASS SPECTROMETRY BASED SECRETOME PROFILING OF HOST RESPONSES TO *RICKETTSIA TYPHI*

Bing Zhu[1], Yingxin Zhao[1], Garrett Cutchin[1], Loka Reddy Velatooru[1], Kamil Khanipov[1], **Rong Fang**[2]

[1]UTMB, Galveston, TX, United States, [2]UTMB, Friendswood, TX, United States

Murine typhus (MT) is an acute and globally distributed infectious disease caused by the obligately intracellular bacterium, *Rickettsia typhi*. Despite its significant public health impact and associated economic burden, the molecular mechanisms driving MT pathogenesis remain poorly defined. In the present study, we applied label-free quantitative mass spectrometry to identify the extracellular and secreted proteins in both cellular and animal models of *R. typhi* infection with a goal to elucidate the molecular basis underlying the development of this disease. Proteomic analysis of the supernatant of *R. typhi*-infected human endothelial cells identified a total of 32 significantly altered host proteins, which are involved in pathways regulating lipid metabolism, inflammation, and immune cell trafficking, and histone modifications. Furthermore, secretome analysis of the serum of lethal animal models of MT revealed 36 differentially regulated proteins compared to sublethal infection. These proteins play a role in initiating the acute phase response, the humoral immune response, and the activation, proliferation, and migration of vascular endothelial cells. Additionally, the severity of MT was found to correlate with upregulated PI3K/AKT signaling, endothelial dysfunction, and a suppressed immune response in myeloid cells, and reduced triacylglycerol and lipid release. These findings may advance an integrative understanding of MT and reveal important host molecules with potential for the development of prognostic markers and countermeasures against the disease.

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RAT BURROWS AND THEIR IMPLICATIONS FOR PLAGUE ECOLOGY

Soanandrasana Rahelinirina[1], Fanohinjanaharinirina Rasoamalala[2], Rija Zafindralambo Andrianarisoa[1], Tojo Rindra Ramihangihajason[3], Florent Sebbane[4], Gauthier Dobigny[5], Mireille Harimalala[3], Minoarisoa Rajerison[1]

[1]Plague Unit, Institut Pasteur de Madagascar, Antananarivo, Madagascar, [2]Simthsonian Institute, Nairobi, Kenya, [3]Medical Entomology Unit, Institut Pasteur de Madagascar, Antananarivo, Madagascar, [4]Institut Pasteur de Lille, Lille, France, [5]Institut de Recherche pour le Développement, Antananarivo, Madagascar

Understanding rat burrow ecology is critical for elucidating mechanisms underlying plague transmission. This study investigates the diversity and seasonal dynamics of flea communities associated with rodent burrows and their hosts in a plague-endemic area of Madagascar. Specifically, this study aimed to characterize the diversity of flea species associated with rodent burrows and their hosts, assess seasonal variations in flea communities between high and low plague transmission periods, and detect the presence of *Yersinia pestis* in rodents and fleas. Sampling was conducted during the high (March) and low (July) plague transmission seasons in 2025, with fleas collected from burrows and directly from small mammals. Spleen and blood samples were collected for rapid diagnosis test (RDT) and serological analysis. Fleas were tested for *Y. pestis* using qPCR and bacteriological culture. Additionally, eight burrows were excavated. A total of fifty-three small mammals were captured in and around the burrows with predominance of *Rattus rattus* and fifty-five fleas were collected from rodents. None of the small mammals tested positive by RDT; however, 23% were seropositive with higher rates in high plague season. All fleas tested negative for *Y. pestis*. Burrows yielded up to 71 adult fleas and some larvae were also collected. Several flea species were identified in burrows across both seasons. Interestingly, the flea community composition remained relatively stable within burrows but varied with the season on rodents with distinct assemblages observed between periods. Notably, flea species found from rodents were also present in burrows, however, flea abondance was higher in burrows than on hosts, suggesting important ecological interactions between hosts and their microhabitats.These finding highlight the key role of burrow environments in maintaining flea populations and potentially sustaining *Y. pestis* transmission throughout the year. Incorporating burrow-level dynamics into surveillance and control strategies could improve our understanding of plague ecology, and therefore the prediction and mitigation of plague outbreaks.

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DIVERSITY OF PARASITES HARBOURED BY COCKROACHES FROM SELECTED PUBLIC ENVIRONMENTS IN ASANTE MAMPONG MUNICIPALITY, GHANA

Mercy Asiedu[1], Agnes Acheampong[2], Samuel Ofori Ayetibo[3], Isaac Adomah[3]

[1]Ghana Education Service, KUMASI, Ghana, [2]Kumasi, AH, Ghana, [3]University of Skills Training and Entrepreneurial Development, KUMASI, Ghana

Cockroaches are recognized as important mechanical vectors of various parasites and pose significant public health risks due to their unsanitary behaviour and ability to harbour diverse microorganisms. This study, therefore, aimed to investigate the spectrum of parasites associated with cockroaches in the Asante Mampong Municipality, Ghana.A total of 116 cockroaches (60.3% *Periplaneta americana* and 39.7% *Blattella germanica*) were collected from hospitals, eateries, marketplaces, toilets, and households using adhesive traps and sterile techniques. Parasitological analysis identified six parasite species: *Enterobius vermicularis* (32.8%), *Ascaris lumbricoides* (25.9%), hookworm (20.7%), *Trichuris trichiura* (12.1%), *Toxoascaris* (5.2%), and *Hymenolepis diminuta* (3.3%). Female cockroaches and *P. americana* exhibited higher parasite prevalence (83.9% and 82.9%, respectively) compared to males (74.1%) and B. *germanica* (73.9%). Hospitals (90.5%) and public toilets (85.0%) recorded the highest parasite prevalence, while households had the lowest (60.0%). Coinfections were common, with 30.4% of positive cockroaches carrying two parasites, and the most frequent combinations included hookworm with *A. lumbricoides* (15.2%) and hookworm with *E. vermicularis* (13.0%). These findings highlight cockroaches as mechanical vectors of medically important parasites, with significant implications for public health in Mampong due to poor sanitation and waste management. The study recommends enhanced

pest control, improved sanitation practices, and public health education to mitigate these risks. Further molecular studies are suggested to confirm parasite species and transmission dynamics.

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DIVERSITY OF PATHOGENIC BACTERIA AND THEIR ANTIMICROBIAL RESISTANCE PROFILES IN COCKROACHES FROM SELECTED PUBLIC ENVIRONMENTS IN ASANTE MAMPONG MUNICIPALITY, GHANA

Vanessa Johnson Bekato[1], Mercy Asiedu[2], Samuel Ofori Ayetibo[3], Agnes Acheampong[4]

[1]University of Energy and Natural Resources, Sunyani, Ghana, [2]Ghana Education Service, KUMASI, AH, Ghana, [3]University of Skills Training and Entrepreneurial Development, Kumasi, Ghana, [4]Kumasi, AH, Ghana

Cockroaches are significant mechanical vectors of pathogenic bacteria and play a role in the dissemination of antimicrobial-resistant organisms within human environments. This study investigated the bacterial carriage and antimicrobial resistance patterns associated with cockroaches collected from hospitals, eateries, markets, households, and toilet facilities in the Mampong Municipality, Ghana. A total of 116 cockroaches were collected, taxonomically identified, and processed using standard microbiological procedures. Antibiotic susceptibility testing of the isolates was performed using the Kirby–Bauer disk diffusion method to determine resistance profiles. Of the 116 cockroaches captured, 60.3% were identified as *Periplaneta americana*, while 39.7% were *Blattella germanica*. The highest numbers were collected from markets and eateries (30 each), whereas households yielded the fewest specimens (n = 15). Bacteriological analysis showed that *Salmonella* spp. were the most prevalent isolates (45%), followed by *Enterococcus* spp. (30%) and *Shigella* spp. (28%). Cockroaches obtained from hospital settings exhibited the highest levels of bacterial carriage, with *Salmonella* detected in 61.9% of samples. Species-specific analysis revealed that *Periplaneta americana* harboured a significantly higher bacterial load compared to *Blattella germanica* (χ^2 = 12.4, df = 1, p = 0.001). Multiple bacterial carriage was common, with over 40% of cockroaches carrying three or more bacterial species. Antimicrobial susceptibility testing indicated high levels of resistance to ampicillin, penicillin, and chloramphenicol. In contrast, ciprofloxacin, gentamicin, amoxicillin, and azithromycin demonstrated comparatively higher efficacy against the isolated organisms.These findings highlight cockroaches as reservoirs of multidrug-resistant bacteria, emphasising the need for improved sanitation, pest control, and antibiotic stewardship in Mampong.

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COMPARING RESULTS FROM ACTIVE AND PASSIVE TICK SURVEILLANCE: IMPLICATIONS FOR *EHRLICHIA* DISTRIBUTION IN SOUTH CAROLINA

Emily Owens Pickle, Madeleine Meyer Torelli, Kia Zellars, Sean Patrick Sweeney, Melissa Nolan

University of South Carolina, Columbia, SC, United States

Tick-borne illnesses are a growing public health concern, and regional awareness of circulating pathogens is critical for assessing disease risk. Surveillance using different sampling approaches can yield distinct patterns that reflect both pathogen ecology and detection methods, with direct implications on human and animal health risks. In this study, we compare *Ehrlichia* spp. detection in ticks using three collection methods: hosting-seeking, public submissions, and animal shelter ticks. From March 2021 to August 2024, 4,165 host-seeking ticks were collected from 47 locations grouped into 1,540 pools by collection, species, life stage, and sex. All host-attached or public submitted ticks are being tested individually; during 2020-2025 3,781 shelter ticks and 323 public tick were submitted. Pools (host-seeking) and individual ticks (public and animal shelter) were tested for *E. chaffeensis, E. ewingii,* and Panola Mountain *Ehrlichia* (PME). Spatial distribution was examined using Getis-Ord Gi* hotspot analysis. *Ehrlichia* DNA was detected in 3.83% (59/1540) of host-seeking pools: *E. chaffeensis* (n=7), *E. ewingii* (n=8), and PME (n=45). All positive pools were from *Amblyomma americanum* ticks, with 50.9% detected in nymphs and collected April-August with notable peaks in mid-spring and late summer. *Ehrlichia* spp. were detected across all four years of surveillance and with substantial clustering at a single collection site. *Ehrlichia* positive ticks were more likely to come from CO_2 baited traps than drags (OR 5.02, CI: 1.93-13.01). Testing of public and shelter ticks is ongoing. Findings from host-seeking ticks suggest that apparent spatial and temporal patterns of *Ehrlichia* may be driven by focal transmission. Ongoing comparisons using passive and active surveillance can explain how collection methods compare and clarify human and animal risk for *Ehrlichia* infection.

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DETECTION OF KELCH 13 IN CYSTS OF *COLPODELLA* SP. (ATCC 50594) BY CONFOCAL MICROSCOPY AND AP2-MU, UBP-1 AND FALCIPAIN-2 DETECTION BY PCR

Tobili Yvonne Sam-Yellowe

Cleveland State University, Cleveland, OH, United States

Colpodella sp. ATCC 50594 is a free-living relative of the pathogenic Apicomplexa such as *Plasmodium* spp., *Babesia* spp. and *Cryptosporidium* spp., causative agents of malaria, babesiosis and cryptosporidiosis, respectively. *Colpodella* species causes colpodellosis through tick bites, biting flies and direct transmission in humans and animals. The mode of transmission, nutrient uptake and pathogenesis in vertebrate hosts are unknown. *Colpodella* sp. ATCC 50594 obtain nutrients by endocytosis and by preying on *Parabodo caudatus* using myzocytosis resulting in the formation of a posterior food vacuole. *Colpodella* spp. have been identified in six different genera of ticks and in a biting fly. However, it is unclear how *Colpodella* species obtain nutrients within the arthropod and vertebrate hosts. Markers of transmission, pathogenesis and nutrient uptake are unknown. Kelch13, coronin, Ap2-mu, Ubp-1 and falcipain-2a genes play important roles in endocytosis in *P. falciparum*. Using polymerase chain reaction (PCR), we previously identified the Kelch13 and coronin genes in *Colpodella* sp. ATCC 50594. The WD repeat motif and Erythrocyte membrane protein 1 was enriched in protein samples from *Colpodella* sp. ATCC 50594 trophozoites analyzed by proteomics. To identify additional genes associated with endocytosis, we amplified the genes for Ap2-mu, Ubp-1 and falcipain-2a from *Colpodella* sp. ATCC 50594 using primers targeting *P. falciparum* genes. Identification of these genes in *Colpodella* sp. (ATCC 50594) demonstrates the conservation of nutrient uptake markers and role of the food vacuole in nutrient uptake.

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A SPECIMEN-LEVEL INTEGRATIVE APPROACH TO RESOLVING SPECIES BOUNDARIES IN SCRUB TYPHUS VECTORS (*LEPTOTROMBIDIUM* GENUS) IN THAILAND.

Kamonchanok Bunmee

Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

Scrub typhus is a significant vector-borne disease in Southeast Asia, transmitted by *Leptotrombidium* chiggers. Accurate identification of these vectors remains a major challenge due to morphological similarity, intraspecific variation, and the presence of cryptic species, limiting our understanding of transmission dynamics and vector diversity. This study applies an integrative taxonomic framework combining molecular and both classical and geometric morphometrics to resolve species boundaries among four *Leptotrombidium* taxa (*L. deliense*, *L. imphalum*, *L. turdicola*, and *L*. cf. *guzhangense*) collected from murine rodents across Thailand. A key advancement of this study is the development of a workflow that enables all analytical approaches to be applied to the same individual specimens, allowing direct comparison between phenotypic and genetic data. Morphological variation was quantified using linear measurements and geometric morphometric approaches, while genetic structure was assessed using mitochondrial (*cox*1) and nuclear (ITS2) markers. Our results demonstrate that geometric morphometric analyses provide taxonomic resolution comparable to classical morphometry, while offering a more

efficient and scalable approach for vector identification. The integration of datasets at the individual level reveals strong concordance between morphometric and molecular patterns, while also uncovering previously unrecognized genetic structuring within *L. deliense*, suggesting the presence of a potential cryptic species. By integrating phenotypic and genetic data from the same specimens, this study provides a robust, reliable, and reproducible framework for species delimitation in chiggers. These findings improve the accuracy of vector identification, enhance our understanding of chigger biodiversity, and strengthen vector surveillance efforts. More broadly, this work demonstrates how integrative, specimen-level approaches can address taxonomic challenges in medically important arthropods, with direct implications for epidemiology and control of vector-borne diseases.

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MAPPING THE INSECTICIDE RESISTANCE LANDSCAPE IN BURKINA FASO (ACROSS 26 SENTINEL SITES): PYRETHROID FAILURE, INTENSITY GRADIENTS, AND METABOLIC DRIVERS IN *ANOPHELES GAMBIAE* S.L.

Charles Nignan[1], Sombié Aboubacar[2], Hyacinthe K. Toe[2], Hien Sawdetuo Aristide[3], Diloma Dieudonné Soma[4], El Hadji Amadou Niang[5], Tiecoura Camara[6], Alphonse Traoré[6], Aïssata Barry[6], Ardjouma Pagbelem[6], Eric Sawadogo[6], Rene Kabore[1], Inessa Ba[1], Roch K. Dabire[3], Diabate Abdoulaye[3], Christian Sidzabda Kompaoré[6], Athanase Badolo[2]

[1]Clinton health Access, Ouagadougou, Burkina Faso, Ouagadougou, Burkina Faso, [2]Université Joseph Ki-Zerbo de Ouagadougou, Burkina Faso, Ouagadougou, Burkina Faso, [3]IRSS, Bobo-Dioulasso, Burkina Faso, [4]Université Nazi Boni, Bobo Dioulasso, Burkina Faso, [5]Université Cheikh Anta Diop, Faculté des Sciences et Techniques Laboratoire d'Ecologie Vectorielle et Parasitaire, Dakar, Senegal, [6]Secrétariat Permanent pour l'élimination du Paludisme (SP/Palu), Ouagadougou, Burkina Faso

Insecticide resistance in *Anopheles gambiae* s.l. threatens pyrethroid-based malaria vector control in Burkina Faso, one of the highest-burden countries in sub-Saharan Africa. Multi-mechanistic resistance data across eco-climatic zones are needed to guide national control strategies. WHO tube tests were conducted at 26 sentinel sites across three eco-climatic zones (September-November 2024) using deltamethrin (0.05%), permethrin (0.75%), malathion (5%), bendiocarb (0.1%), and pirimiphos-methyl (170 mg/m²). Resistance intensity assays and PBO synergist bioassays were performed at selected sites. Species identification and kdr-West (L1014F) and kdr-East (L1014S) genotyping were conducted by PCR and TaqMan assays on 548 mosquitoes. Confirmed pyrethroid resistance was detected at all 26 sites, with no significant inter-zonal variation (Kruskal-Wallis, $P > 0.05$). Resistance intensity was high at Kaya (RR = 18.7, 95% CI: 11.2-31.4) and moderate at Ouagadougou (RR = 7.1). Malathion and pirimiphos-methyl retained high efficacy (mortality ≥ 95% at most sites); confirmed bendiocarb resistance was limited to Diébougou (89.1%, $P = 0.013$). PBO restored pyrethroid susceptibility at 9 of 14 combinations ($P < 0.05$), with full restoration at four sites (SR ≥ 3.1), confirming widespread CYP450-mediated metabolic resistance. The kdr-W allele was not correlated with deltamethrin mortality (Spearman's $\rho = -0.275$, $P = 0.301$), confirming metabolic mechanisms as primary drivers. In conclusion, *An. gambiae* s.l. in Burkina Faso displays severe, uniform, multi-mechanistic pyrethroid resistance. Organophosphates retain efficacy and warrant protection through IRS rotation. PBO-pyrethroid nets should be deployed as a priority.

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GENOMIC EVIDENCE OF ADAPTATION IN THE OXIDATIVE STRESS PATHWAY OF *ANOPHELES COLUZZII* FROM GHANA

Jessica Arthur[1], Enock Kofi Amoako[2], Mona-Liza Ebiere Sakyi[3], Collins Misita Morang'a[4], Dominic Selorm Yao Amuzu[5], Gordon Awandare[6], Lucas Naam-Kayagre Amenga-Etego[7]

[1]West African Centre for Cell Biology of Infections Pathogens, Legon, Ghana, [2]West African Centre for Cell Biology of Infectious Pathogens, University of Ghana, Accra, AA, Ghana, [3]West Africa Centre for Cell Biology of Infectious Pathogens, Accra, AA, Ghana, [4]WACCBIP, Accra, Ghana, [5]WACCBIP, Dept of Biochemistry, University of Ghana, Accra, Accra, Ghana, [6]University Of Ghana, Legon, Ghana, [7]University Of Ghana, Accra, TV, Ghana

Insecticide resistance remains a major threat to malaria vector control across sub-Saharan Africa, with oxidative stress-mediated detoxification increasingly implicated as a contributing mechanism. The Kelch-like ECH-associated protein 1 (Keap1) gene is a central repressor of Nrf2-mediated detoxification, and variants disrupting this function may contribute to resistance advantage in exposed mosquito populations. This study sought to determine whether Keap1 shows signatures of adaptive evolution in *Anopheles coluzzii* populations in Ghana.We analysed whole-genome sequencing data from 2,073 *An. coluzzii* samples across six Ghanaian regions, from the MalariaGEN dataset. We quantified coding variant frequencies at the Keap1 locus, assessed gene copy number variation, examined population structure, and applied H12 haplotype homozygosity scanning to detect signatures of selection.Of 2,427 coding variants identified, 1,654 were non-synonymous, of which eight exceeded 50% allele frequency across cohorts. These high-frequency variants, including A895G (63-96%), S734N (78-97%), and T960M (45-92%), were concentrated in the exon regions. Gene copy number deletions were equally pervasive, detected at 67-82% frequency across all regions. Population structure analysis revealed moderate differentiation among cohorts, with the Greater Accra Region as the most divergent (FST = 0.203). H12 scanning identified a pronounced haplotype homozygosity signal at the Keap1 locus across multiple regions, consistent with positive selection.The co-occurrence of high-frequency non-synonymous variants, widespread gene deletions, and a strong selection signal collectively supports adaptive evolution at the Keap1 locus in *An. coluzzii* in Ghana. These findings are consistent with insecticide-driven selective pressure acting through the oxidative stress pathway, positioning Keap1 as a candidate resistance locus warranting further functional characterisation.

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TWO HIGHLY SELECTED CYTOCHROME *6P4A* AND *6P4B* ALLELES CONFER INSECTICIDE RESISTANCE IN THE MAJOR MALARIA VECTOR *ANOPHELES FUNESTUS* IN EAST AND CENTRAL AFRICA AND REDUCE THE EFFECTIVENESS OF INSECTICIDE-TREATED BED NETS

Nelly Manuela Tchatchoua Tatchou[1], Riccado F. Thiomela[1], Mahamat Gadji[1], Derrick Fofie[1], Fleuriane M.D Kamga[1], Carlos Djoko Tagne[1], Abdullahi Muhammad[2], Charles S. Wondji[3]

[1]Centre for Research in Infectious Diseases, Yaounde, Cameroon, [2]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [3]Liverpool Sch of Tropical Medicine, Liverpool, United Kingdom

The rise of insecticide resistance (IR) in malaria vectors is a severe threat to malaria control efforts. Understanding the underlying mechanisms of IR is key for applying effective vector control strategies. Our study deciphered how two genes, *Cytochrome 6P4a* and *6P4b,* drive IR in the major malaria vector, *Anopheles funestus, in East and Central Africa*. We used whole genome sequencing (WGS) on highly resistant and highly susceptible *An. funestus* from Cameroon sampled in 2021; insecticide metabolism assays; and transgenic Drosophila fly models to identify and characterise the genetic drivers of pyrethroid IR in this population. We further designed two field-deployable DNA-based molecular diagnostic tools to detect and monitor IR in the field. The WGS unveiled genomic signatures linked to strong resistance at the *rp1* (chromosome 2R) and *CYP9* (chromosome X*)* loci, with the selection of two mutant *CYP6P4a* and *CYP6P4b* gene variants. Enzymes from these variants exhibited significantly greater ability (3-fold) to metabolise pyrethroid insecticides compared to the wild-type enzymes. Also, expression of these variants in transgenic flies conferred significant IR compared to those expressing the wild-type alleles (mortality <50% vs. >80%, respectively), confirming the role of *6P4a* and *6P4b* in inducing IR. In addition, our molecular diagnostic tools linked these variants to reduced bed net efficacy, as mutant mosquitoes showed higher survival after exposure to deltamethrin- and alphacypermethrin-treated bed nets,

increasing their lifespan, hence likely contributing to sustaining malaria transmission. Also, using the tools, tracking the spatial and temporal spread of these variants showed an alarming rise throughout many regions in Cameroon, from less than 10% frequency in 2014 to 98% in 2021. A broader assessment across Africa showed that these variants are spread in *An. funestus* across East and Central Africa. Overall, our study established that *CYP6P4a* and *CYP6P4b* play a key role in the high IR in the malaria vector *An. funestus* and introduced two DNA-based IR diagnostic tools to strengthen the IR monitoring and management toolbox in Africa.

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EVOLUTION AND SPREAD OF INSECTICIDE RESISTANCE VARIANTS IN *ANOPHELES GAMBIAE* S.L. FROM BURKINA FASO

Mahamadi Kientega[1], Honorine Kaboré[1], Grégoire Sawadogo[1], Nouhoun Traoré[1], Tin-Yu J. Hui[2], Abdoul-Azize Millogo[1], Hamidou Maiga[1], Alistair Miles[3], Chris. S. Clarkson[4], Abdoulaye Diabaté[1]

[1]Institut de Recherche En Sciences de La Santé (IRSS/DRO), Bobo-dioulasso, Burkina Faso, [2]Department of Life Sciences, Imperial College London, Silwood Park, Ascot, SL5 7PY, UK, London, United Kingdom, [3]Wellcome Sanger Institute, Cambridge, United Kingdom, [4]Liverpool school of tropical medicine, Liverpool, United Kingdom

The intensive use of insecticide-based control tools has led to the rapid evolution of resistant phenotypes in malaria vector populations. Understanding the evolutionary processes underlying these resistances is essential to inform the development and deployment of effective control interventions. This study investigated the geographical spread and the genetic background of insecticide resistance variants in *Anopheles gambiae* s.l. in Burkina Faso. The study identified five pyrethroid-resistant mutations (*995F, 995S, 402L(g>t,c), 1527T* and *1570Y)* at high frequencies. Six diplotype groups were identified, including novel combinations of the resistance-associated alleles (*995F, 402L(g>t,c)* and *1527T*), which formed new genotypes within *An. coluzzii* populations. These results suggest the emergence of new resistance genotypes in *An. coluzzii* that are not associated with 995F, probably due to recombination and gene flow events. Interestingly, strong linkage disequilibrium (r^2*=0.821*) was observed between *1527T* and *402L(g>t)* compared to *1527T* and *402L(g>c)*. The PCA revealed three clusters of *An. coluzzii* populations, driven by *995F, 402L(g>t,c)* and *1527T*. Other insecticide resistance associated variants such as copy number variations and SNPs in the *Ace1* gene (*ace1-G280S*), cytochrome P450s, esterases and glutathione S-transferases were identified at high frequencies in the same mosquito populations, indicating the intensity and diversity of resistance mechanisms in the country. The study underscores the extent and spreads of insecticide resistance variants in Burkina Faso. It highlights the importance of genomic surveillance of malaria vectors to monitor and detect new resistance variants and to understand the evolutionary processes in vector populations.

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DECLINING *KDR* ALLELE FREQUENCIES IN AN EMERGING *AEDES AEGYPTI* POPULATION IN ALBUQUERQUE, NEW MEXICO

Miriam A. Bechtel

Center for Global Health, University of New Mexico, Albuquerque, NM, United States

Aedes aegypti, an invasive mosquito species, was first detected in the Albuquerque, New Mexico metropolitan area in 2018 and is now locally established. Control of adult *Ae. aegypti* populations rely predominantly on pyrethroid-based insecticides; however, resistance to these compounds is widespread. Pyrethroid resistance in *Ae. aegypti* is associated with non-synonymous single-nucleotide polymorphisms (SNPs) in the voltage-gated sodium channel (*VGSC*) gene, commonly referred to as knockdown resistance (kdr) mutations. In this study, the longitudinal prevalence of three VGSC kdr mutations, V410L, V1016I, and F1534C, in *Ae. aegypti* collected in Albuquerque between 2018 and 2024 (n=409) was assessed. Genomic DNA isolated from individual mosquitoes was genotyped at each SNP locus using allele-specific quantitative PCR. Haplotypes were inferred using PHASE v2.1.1. The frequency of the triple-mutant haplotype (410L-1016I-1534C) declined from near fixation in 2018-2019 (0.99) to 0.85 in 2024 (bootstrap trend test: $p < 0.001$). These results suggest reduced selection for pyrethroid target-site resistance in the Albuquerque *Ae. aegypti* population. This decline coincided with documented changes in insecticide use beginning in 2022. The observed reduction in the triple-mutant haplotype is also consistent with possible fitness costs associated with resistance-linked VGSC mutations. Together, these findings provide important context for interpreting kdr allele frequencies in this newly established *Ae. aegypti* population in Albuquerque, New Mexico.

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DISTRIBUTION OF DETOXIFICATION GENES INVOLVED IN METABOLIC RESISTANCE IN THE TWO MAIN MALARIA VECTORS, *ANOPHELES GAMBIAE S.S.* AND *AN. COLUZZII,* THROUGH A TRANSECT SOUTH-NORTH IN BENIN FOR DECISION MAKING

Martin Akogbeto Codjo[1], Arsene Fassinou[1], Aurore Hounto[2], Salako Luc Djogbenou[3], Razaki Osse[4], Juvenal Ahouandjinou[1], Linda Towakinou[1], Filémon Tokponnon[1], Louisa Alexandra Messenger[5], Germain Gil Padonou[6]

[1]CREC Ministry of Health, Cotonou, Benin, [2]Institut de Recherche Clinique du Bénin (IRCB), Abomey-Calavi, Benin, [3]Tropical Infectious Diseases research Center/ University of Abomey-Calavi, Ouidah, Benin, [4]CREC, [5]University of Nevada, Las Vegas, Henderson, NV, United States, [6]Ministry of Health, Cotonou, Benin

World Health Organization (WHO) recommended to use bitreated pyrethroid-PBO Long Lasting Insecticidal Nets (LLIN) in areas where mosquito vectors are resistant to pyrethroids. But the cost of PBO-LLINs is high and the quantity purchased each year in Benin is not sufficient to cover the entire country. The NMCP therefore proposed to identify the communes with high metabolic resistance to be favored when distributing PBO nets. The study was carried out in 24 communes to cover all agroecological zones, with two communes per department. Populations of *Anopheles gambiae* s.l. from the 24 communes were subjected to susceptibility tests according to WHO and CDC methods combined with the PBO synergist to confirm their resistance to pyrethroids and to check the involvement of metabolic resistance in these vectors. Legs and wings of the same mosquito were molecularly speciated using the protocol of Santolamazza *et al*. (2008) to identify the presence of populations of *An. gambiae* and *An. coluzzii*. Involving of overexpression enzyme genes in metabolic resistance was analyzed by the distribution of detoxification genes involved by quantitative PCR (qPCR) using Sybr. Eight (08) different enzyme genes involved in metabolic resistance were characterized in the 24 communes. The frequency of the most genes (Cyp6M2, Cyp6P4, Cyp6P1, Cyp6Z1) varied from 33% to 42%. Three genes (Cyp9K1, Cyp6P3 and GstE2) represented 20%-30% followed by Cyp6G16 (8%). Sixteen (16) Communes are characterized by a low level of enzyme detoxification genes and are priority for the allocation of PBO-ITNs. At the same time, the rate of inhibition of vector resistance by the PBO synergist is high (>50%) in 16 communes which are good candidates for the allocation of PBO-ITNs. Combining the two lists, 20 communes are eligible for the allocation of PBO-TNs among the 24. This study aims to identify the priority departments to receive PBO-LLINs based on the results of the level of enzyme detoxification genes observed in each commune. For now, 7 departments were identified. The study should continue to clarify the situation of the other 5 departments.

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INTEGRATED DENGUE VIRUS AND GENETIC INSECTICIDE RESISTANCE SURVEILLANCE IN *AEDES AEGYPTI* FROM IQUITOS, PERU

Fanny Castro-Llanos[1], Marisa Lozano[1], Helvio Astete[1], Victor M. Lopez[1], Karin Escobedo-Vargas[1], Beyquer Zambrano[1], Luz A. Romero[1], Steev Loyola[1], Jorge A. Cime[2], Carmen Flores-Mendoza[1], Ryan C. Moore[1], Gissella Vasquez[1]

[1]U.S. Naval Medical Research Unit SOUTH, Bellavista, Peru, [2]National Institute of Health, Cuernavaca, Mexico

The largest dengue outbreak in the Americas was in 2024 with around 13 million cases. In Peru, the 2023 and 2024 dengue outbreaks exceeded 250,000 cases. Iquitos, a city in the Peruvian Amazon, has experienced multiple dengue outbreaks in the last years. *Aedes aegypti* is the main vector of dengue, chikungunya and Zika viruses. Insecticide spraying remains the most effective measure to interrupt *Aedes*-borne disease transmission; however, overuse has led to the emergence and spread of vector resistance. Pyrethroid resistance in *Ae. aegypti* has been associated with knockdown resistance (*kdr*) mutations (V1016I, F1534C). The aim of this study was to conduct integrated surveillance of DENV infection and *kdr* mutations in *Ae. aegypti* from dengue hotspot sites in Iquitos. Mosquito collections were performed at 134 sites in Iquitos city, Loreto state, from Sep-2022 to Feb-2025 using prokopack aspirators. *Aedes aegypti* samples were selected for DENV testing based on Peruvian government dengue case reports. Individual mosquito head/thorax were screened using DENV/ZIKV/CHIKV qRT-PCR. Specimens (legs) from mosquito DENV positive sites were screened for *kdr* mutations using allele-specific PCR. Bivariate statistical analyses were performed to assess associations between dengue incidence rate, mosquito DENV infection, and *kdr* genotype frequencies. A total of 12,205 *Ae. aegypti* females were collected; 1,205 (9.9%) specimens from 33 sites with high dengue incidence were screened for arboviruses. DENV was detected in 13 mosquitoes from 5 sites across Iquitos with infection rates (IR) ranging from 1.7% to 14.3%. A subset of 357 mosquitoes from these sites was tested for *kdr* mutations. Four genotypes were identified (IICC, VICC, VVCC, VVFF), with moderate frequencies (22.5-58.2%) of high resistance genotypes (VICC, IICC) suggesting resistance to pyrethroids. Dengue incidence rate and mosquito DENV IR were positively correlated ($P<0.001$). No association was found between mosquito DENV IR and *kdr* genotype frequencies. Our findings support the value of entomo-virological surveillance for improving dengue risk assessment and outbreak forecasting.

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DETECTION AND DISTRIBUTION OF KDR MUTATION L1014F CONFERRING PYRETHROID RESISTANCE IN *CULEX PIPIENS* COLLECTED IN KISUMU COUNTY, KENYA

Gladys Kerich[1], JANET Ambale[2], Jane Wambui Thiiru[3], Jacob Ngugi[1], Santos J. Yalwala[4], David Ochieng Oullo[5], Nicholas Okoth Odemba[6], Francis Ngere[1], Richard Odinga Ochieng'[2], Eunice Achieng[1], Lucy Isabella[1], Elizabeth Kioko[7], Joel Lutomiah[8], John Eads[9]

[1]KEMRI/WRAIR-A, KISUMU, Kenya, [2]USAMRDA-K, Kisumu, Kenya, [3]Kisumu, Kenya, [4]US Army Med Res Unit-Kenya, Kisumu, Kenya, [5]HJFMRI, Kisumu, Kenya, [6]USAMRDA - K, Kisumu, Kenya, [7]USAMRU-K (WALTER REED PROJECT), Kisumu, Kenya, [8]Kenya Medical Research Institute (KEMRI), Nairobi, Kenya, [9]DPO, AE, United States

Culex pipiens complex comprises of *Cx. pipiens* and *Cx. quinquefasciatus* species which are vectors of West Nile virus (WNV). Mosquitoes breeding in polluted habitats are more likely to survive insecticide applications, thus compromising control efforts. Pyrethroids, the only recommended insecticides for treated nets, are widely used to control adult mosquitoes. However, prolonged usage has led to insecticide resistance among mosquito populations. This study sought to investigate the prevalence of kdr mutations in *Cx. pipiens* complex. Larvae were collected in carwash areas using standard dippers, transported to the insectary at Kisian campus and reared to F0 colonies. 3-5-day old females were subjected to WHO bioassays using permethrin (0.75%), PBO (4%) and permethrin (0.75%) and deltamethrin (0.5%) with controls exposed to untreated papers. The number of knockdown mosquitoes were recorded at 10, 15, 20, 30, 40, 50 and 60 minutes post-exposure. Mortality rates were scored after 24h post-exposure. DNA was extracted mosquitoes using Qiagen kit and kdr mutations detected by real time PCR. The three insecticide exposures depicted varying levels of resistance with permethrin observed mortality of (8%), PBO and permethrin (18%) and deltamethrin (63%). The frequency of L1014F mutation in permethrin-resistant mosquitoes was homozygous wildtype (47.3%), heterozygous mutants (47.30%) and homozygous mutants (5.4%). For deltamethrin, homozygous wildtypes were 44.20%, heterozygous mutants (51.45%) and homozygous mutants (4.35%). The synergist PBO and permethrin exposures had 74.08% homozygous wildtypes and 25.92% heterozygous mutants. The overall allele frequency of L1014F mutation was higher in permethrin-exposed mosquitoes suggesting increased resistance compared to deltamethrin, and PBO and permethrin which had the least allele frequency. Pre-exposure to PBO increased mortality compared to exposure to permethrin alone, suggesting that PBO enhances the efficacy of permethrin. Continuous monitoring of resistance alleles is critical in planning and implementing better vector control strategies.

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HIGH EFFICACY OF ATTRACTIVE TARGETED SUGAR BAITS (ATSB) AGAINST INSECTICIDE-RESISTANT *ANOPHELES ARABIENSIS* IN MALI

Amadou Sékou Traore[1], Mohamed Moumine Traore[1], Boubacar Sow[1], Younoussa Ziguime[1], Aboubakr Sadik Kone[1], Gaoussou Fofana[1], Cheick Oumar Tangara[1], Sophie Sarrassat[2], Mahamoudou TOURE[1], Mahamadou DIAKITE[1], Immo Kleinschmidt[2], Seydou Doumbia[1], Günter C. Müller[3]

[1]University Clinical Research Center (UCRC) University of Sciences, Techniques and Technologies of Bamako (USTTB), Bamako, Mali, [2]London School of Hygiene and Tropical Medicine, London, United Kingdom, [3]Kuvin Center for the Study of Infectious and Tropical Diseases, Hadassah Medical School, The Hebrew University, Jerusalem, Israel

The World Health Organization has called for new vector control tools to address rising insecticide resistance and residual malaria transmission. Attractive Targeted Sugar Baits (ATSB) exploit mosquito sugar-feeding behavior to kill vector mosquitoes, but susceptibility to the active ingredient dinotefuran of pyrethroid resistant genotypes remains unclear. We conducted experimental bioassays to assess dinotefuran-based ATSB efficacy against knockdown resistant (kdr) genotypes (RR, RS, SS) of *Anopheles arabiensis* (F2 generation). Mortality was measured at 24- and 48 hours post-exposure and stratified by genotype and sex. ATSB showed high efficacy across all genotypes. At 24 hours, mortality was ≥96% for RR, RS, and SS mosquitoes, reaching 100% (N=300) at 48 hours. Among RR genotypes, mortality at 24 hours was 100% in males and 92% in females, with both sexes reaching 100% at 48 hours. For RS genotypes, mortality was 100% in males and 98% in females at 24 hours, and 100% for both sexes at 48 hours. SS mosquitoes showed 100% mortality at both time points. Overall, ATSB efficacy exceeded 95% regardless of genotype or sex. Dinotefuran-based ATSB is highly effective against *An. arabiensis*, including pyrethroid-resistant genotypes, confirming its potential as a complementary tool for malaria vector control. By targeting sugar-feeding behavior, ATSB can overcome limitations of conventional insecticide-based interventions and address residual transmission. These findings provide strong evidence to support ATSB integration into malaria control programs, particularly in regions facing widespread insecticide resistance.

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EVIDENCE OF INSECTICIDE RESISTANCE IN *AEDES AEGYPTI* POPULATIONS OF SURINAME FIRST REPORT OF MALATHION RESISTANCE AND PRESENCE OF THE *KDR* MUTATIONS V410L, V1016I AND F1534C

Kartika Devi Doerdjan, Jerry Toelsie

Anton de Kom University of Suriname, suriname, Suriname

Dengue and other arboviral diseases remain a significant public health concern in Suriname, where vector control relies heavily on chemical insecticides. However, limited data on insecticide resistance hinder the effectiveness of these interventions. This study assessed the resistance status of Aedes aegypti populations from multiple locations in Suriname using CDC bottle bioassays and investigated underlying mechanisms through molecular detection of knockdown resistance (kdr) mutations (V410L, V1016I, and F1534C) using TaqMan genotyping assays. Results provide the first evidence of malathion resistance in Suriname with confirmed resistance in one population and suspected resistance in others. Strong resistance to lambda cyhalothrin was observed across all tested locations. High frequencies of resistance-associated alleles were detected, with 410L and 1016I approaching fixation and 1534C nearly fixed in the population. These findings demonstrate widespread insecticide resistance in Aedes aegypti in Suriname and highlight the role of kdr mutations in shaping resistance profiles. The study provides unique genetic data for a previously underrepresented region and has direct implications for vector control strategies, particularly in guiding insecticide selection and resistance management.

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EVALUATING THE EFFECTS OF CHLORFENAPYR EXPOSURE ON PYRETHROID RESISTANT MOSQUITOES

Sabaa Logman[1], Daniel Hartmen[2], Dieunel Derilus[1], Lucy Mackenzie Impoinvil[1], Lisa Reimer[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Washington State Department of Health, Tumwater, WA, United States

Pyrethroid-based insecticides are widely used in malaria and arbovirus control. However, growing pyrethroid resistance is compromising effectiveness, posing a significant public health threat. Chlorfenapyr, a novel pyrrole pro-insecticide, has been introduced to target pyrethroid-resistant mosquitoes due to its unique mode of action. It is activated by oxidases, which are protective against pyrethroids, and the oxidated compound inhibits the mitochondrial electron transport chain. To explore the relationship between pyrethroid resistance and chlorfenapyr efficacy, we conducted a series of experiments with pyrethroid resistant *Aedes aegypti* and *Anopheles gambiae* with the objective of informing future vector management strategies. Pyrethroid resistant *Anopheles gambiae* Siaya strain from western Kenya were exposed to a chlorfenapyr dose of 40µg or 60µg every generation or every other generation, respectively, for six selections over a span of seven months. Resistance to chlorfenapyr and permethrin was compared between the selected and the unselected strains. We observed a significant increase in survival of the selected strain to low doses of chlorfenapyr with no change in the levels of permethrin resistance. We also examined the impact of sub-lethal chlorfenapyr exposure on fecundity and development. We observed no significant differences in overall fecundity between the exposed and unexposed mosquitoes, however there were slight changes at different developmental stages. Finally, we utilized RNA sequencing to determine transcriptional changes associated with chlorfenapyr exposure at different time points. Taken together, these studies explore the complicated mode of action of chlorfenapyr, the long-term effects of utilizing chlorfenapyr in pyrethroid resistant populations and the potential for cross resistance.

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INVASION OF *AEDES ALBOPICTUS* IN GHANA: ECOLOGY AND INSECTICIDE RESISTANCE STATUS

Faustina A. Owusu, Christopher M. Owusu-Asenso, Anisa O. Abdulai, Isaac K. Sraku, Akua O. Forson, **Yaw A. Afrane**

University of Ghana, Accra, Ghana

In mid-2023, *Aedes albopictus*, a key dengue vector, was unexpectedly found breeding in huge numbers during *Anopheles* surveillance in Takoradi, a seaport city in southwestern Ghana. *Ae. albopictus* were previously identified sparingly breeding in Accra, Ghana until this encounter. By mid-2024, the Ghana Health Service reported several outbreaks of dengue for the first time, with confirmed cases in several areas, including Takoradi. This study investigated the bionomics and insecticide susceptibility of *Ae. albopictus* through larval and adult surveys near the initial detection sites, and its spread into the forest areas. Larval surveys were conducted in 2023/2024 by sampling *Aedes* immatures from water-holding containers close to human settlements. Adult *Aedes* mosquitoes were sampled using the Biogents Sentinel (BG) traps, Human Landing Catch (HLC) and Prokopack (PPK) aspirators. Among 12,666 *Aedes* larvae collected, car tyres were the most productive habitat (66.4%). *Ae. aegypti* (87.2%) were the most abundant vector, followed by *Ae. albopictus* (12.2%). Follow-up surveys conducted two years later revealed that *Ae. albopictus* had expanded from the coastal savannah areas, where it was initially detected, into the forest zones. *Ae. albopictus* was susceptible to pyrethroids [mortality rate (MR) = 98.8–100%] and pirimiphos-methyl [MR = 100%], while *Ae. aegypti* was resistant to pyrethroids [MR = 74.3-75%]. PBO synergist assays restored susceptibility in *Ae. aegypti*. *kdr* mutations were detected in both species: *Ae. albopictus* had low frequencies of *F1534C* (0.18), *V410L* (0.02), *V1016I* (0.00) whilst *Ae. aegypti* showed high *F1534C* (0.72), *V1016I* (0.50), and *V410L* (0.06). These findings provide essential baseline data for public health action and necessitate the urgent need for enhanced vector surveillance and resistance monitoring in Ghana.

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INVESTIGATION OF INSECTICIDE RESISTANCE PHENOTYPES IN DIAPAUSING *CULEX PIPIENS* MOSQUITOES

Donald Ward, Heather Jaurigui, Silvie Huijben

Arizona State University, Tempe, AZ, United States

Insecticide resistance has become an excessive burden on the ability to control mosquito vectors of tropical diseases. Resistance phenotypes vary widely in response to different insecticides and with respect to other factors including species, temperature, humidity, and molecular and anatomical resistance mechanisms. Further, resistance mechanism activity has been shown to fluctuate seasonally in *Culex* and *Anopheles* spp., a phenomenon which could be brought about by reduction in insecticidal selection pressures in seasons of low mosquito abundance. Moreover, certain species of mosquitoes and other insects in more temperate climates undergo diapause, a physiological change of low metabolic activity. The impact of diapause on insecticide susceptibility, especially when resistance is affected by the activity of detoxifying metabolic processes, has not been studied in mosquitoes. Given that diapause reduces metabolic activity, we hypothesize that diapause reduces resistance to insecticides, particularly organophosphates. To test this, we assessed the LD_{50} of non-diapausing and diapausing *Culex pipiens* mosquitoes at three different temperatures and two different photoperiods for both deltamethrin (pyrethroid) and malathion (organophosphate). Diapause was induced by incubation of larvae starting at the L2 stage with an 8:16 (Light:Dark) photoperiod and exposure to 18°C and 80% RH until 7 days post-eclosion. Seven-day-old adults were transferred to 10, 18, or 26°C until 14 and 30 days post-eclosion, at which point topical application bioassays were performed to assess resistance phenotype. Early data show reduction in LD_{50} in diapausing mosquitoes over time at 26°C when exposed to deltamethrin. However, an increase in LD_{50} was found when mosquitoes were exposed to malathion at 26°C. Knowledge about the diapause status in *Cx. pipiens*

could allow vector control programs to target mosquito populations at specific times of year to overcome resistance and to reduce transmission of tropical diseases that circulate in non-tropical climates due to climate change and vector range expansion.

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CIRCUMSPOROZOITE PROTEIN RATES, AND GENETIC MUTATIONS IN WILD POPULATIONS OF *ANOPHELES* SPP. IN TWO ECOLOGICAL ZONES IN GHANA

Thomas Kwame Addison[1], Kingsley Badu[2], Addo Kofi Agyapong[1], Austine Tweneboah[1], Ackom Abbas Dawood[1], Mutala Abdul-Hakim[1], Stephen Opoku Afriyie[3]

[1]KNUST, KUMASI, Ghana, [2]Kumasi, AH, Ghana, [3]Texas Tech University, Lubbock, TX, United States

Malaria transmission in Ghana varies across ecological zones, shaped by vector diversity and the spread of insecticide resistance. Understanding how *Anopheles* species composition, biting behavior, and resistance alleles influence local transmission is essential for guiding targeted control strategies. The study was conducted in two ecological zones of Ghana: Afamanso in the Ashanti Region and Obom in the Greater Accra Region. Quarterly entomological surveys were performed during rainy and dry seasons. Adult mosquitoes were collected indoors and outdoors using human landing catches (HLC). Specimens were morphologically identified and *Plasmodium falciparum* sporozoite infections were detected using circumsporozoite protein ELISA. Molecular PCR assays were used for species identification within the *An. gambiae* complex. Allele-specific PCR was employed to detect knockdown resistance (kdr-East and kdr-West) and acetylcholinesterase-1 (Ace-1) mutations. Entomological inoculation rate (EIR), were calculated to assess transmission intensity. A total of 8,022 *Anopheles* mosquitoes were collected across the two zones. *An. gambiae s.l.* was the predominant vector, with *An. Coluzzii (*47.5 %*)* and *An. gambiae s.s (*52.5 %*).* most frequent, while *An. arabiensis* and *An. melas* were not present. Sporozoite ELISA confirmed *P. falciparum* infections in both zones, with higher infection prevalence in Obom. HBR and EIR were significantly higher in Obom compared to Afamanso. Molecular genotyping revealed widespread kdr mutations, with kdr-East more frequent in Obom and kdr-West dominant in Afamanso. Ace-1 mutations were detected at lower frequencies but often co-occurred with kdr alleles, indicating multiple resistance mechanisms in circulation. In conclusion**, m**alaria transmission in Ghana is influenced by ecological differences in vector diversity, biting behavior, and insecticide resistance. The co-occurrence of kdr and Ace-1 mutations highlights an emerging challenge for vector control. Continuous surveillance is critical to sustain malaria control and support elimination goals.

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SPATIOTEMPORAL MODELING OF CLIMATE AND SPATIAL DRIVERS OF DENGUE TRANSMISSION ACROSS MEXICAN MUNICIPALITIES

Oluwatosin Babasola

University of Georgia, Athens, GA, United States

Dengue is one of the fastest growing mosquito borne viral diseases worldwide, with increasing epidemic activity across tropical and subtropical regions. In Mexico, transmission follows marked seasonal patterns and varies substantially across municipalities. Climatic conditions affect mosquito abundance and viral replication; however, persistent spatial clustering suggests that transmission is also shaped by underlying geographic and structural factors. For this reason, understanding the combined effects of climatic variability and spatial dependence is important for strengthening early warning systems and improving vector control. This study examined the spatiotemporal dynamics of dengue incidence in Mexico and quantified the contribution of climatic variability to observed transmission patterns. Municipality level dengue cases reported from 2020 to 2025 were analyzed within a spatiotemporal framework. Weekly surveillance data were linked to temperature, rainfall, and relative humidity. Moran's I statistics were used to assess spatial autocorrelation and identify clusters of elevated transmission. Associations between climatic variables and dengue incidence were estimated using Bayesian negative binomial regression models. The results showed persistent clustering of dengue incidence across municipalities, with significant positive spatial autocorrelation in each study year. High risk clusters were concentrated in southern and coastal regions. Temperature had the strongest positive association with dengue incidence, whereas rainfall and relative humidity showed weaker and less consistent effects. Residual spatial variation remained after adjustment for climatic factors, indicating that climate alone did not fully explain the geographic distribution of dengue transmission. These findings show that both climatic variability and persistent spatial structure contribute to dengue transmission in Mexico. Accordingly, surveillance and control strategies should account for both climatic indicators and spatial risk information

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SUSCEPTIBILITY PROFILES OF *CULEX QUINQUEFASCIATUS* AND *AEDES AEGYPTI* MOSQUITOES IN MARICOPA COUNTY, AZ

Ernest Boampong, Ryon Salek, Amareet Singh, Silvie Huijben

Arizona State University, Tempe, AZ, United States

The southwestern United States has emerged as a hotspot for West Nile virus activity. In 2021, Maricopa County experienced the largest WNV outbreak ever recorded in a single U.S. county. Pyrethroid insecticides are the primary vector control tool, but widespread resistance has compromised efficacy. We investigated susceptibility profiles in *Culex quinquefasciatus* and *Aedes aegypti* across the greater Phoenix area. Field-collected mosquitoes were sampled from three randomly selected sites, each containing *Cx. quinquefasciatus* and *Ae. aegypti.* CDC bottle bioassays were used to determine susceptibility to permethrin, deltamethrin, etofenprox, and malathion. Piperonyl butoxide (PBO) synergist assays were also tested according to CDC guidelines. *Cx. quinquefasciatus* and *Ae. aegypti* exhibited resistance to permethrin, deltamethrin, and etofenprox in all sites. PBO co-exposure significantly increased *Culex* mortality, indicating metabolic detoxification as the possible primary mechanism. In *Aedes*, minimal mortality recovery post PBO exposure suggests target-site mutations (kdr) may be the predominant resistance mechanism. Malathion remained fully susceptible across all sites.This study documents widespread, mechanistically distinct pyrethroid resistance in both vectors. The divergent metabolic mechanisms in *Culex* and the target site in *Aedes* require species-specific strategies. Malathion offers an alternative after a decade of non-use. Our findings underscore the urgent need for an integrated resistance management plan in Maricopa County that rotates insecticide classes, incorporates synergists, and tailors strategies to local resistance profiles. Without proactive implementation, vector control agencies risk losing critical tools for preventing arboviral disease outbreaks in the Pacific West.

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MOSQUITO SURVEILLANCE ACROSS HABITATS IN MYANMAR

Austin J. Mejia[1], Ismaaeel Abdur-Rahmaan[1], Ohnmar Aung[2], Nang Phyu Phwe[3], Pyae Phyo Aung[3], Thura Soe Min Htike[3], Tin Htun Aung[3], Juliette Lewis[1], Nicole Gardner[2], Tierra Evans[4], Christine Krueder Johnson[2], Rebekah Kading[1]

[1]Colorado State University, Fort Collins, CO, United States, [2]UC Davis School of Veterinary Medicine, Davis, CA, United States, [3]Nature Conservation Society, Myanmar, Myanmar, [4]Berkeley, CA, United States

Myanmar is considered one of the most biodiverse countries in the Asia-Pacific region. Yet, its mosquito population and role in arbovirus transmission are greatly understudied. To address this knowledge gap, we conducted mosquito surveillance in Myanmar across a landscape gradient consisting of edge and urban habitats. We hypothesize that virus-host plasticity increases at edge habitats where vertebrates and mosquitoes

interact. We collected 36,194 mosquitoes from edge and urban habitats across the wet and dry seasons and will be analyzing species diversity, bloodmeal source, and arbovirus presence. Preliminary data consists of 50 mosquito species across 10 genera that feed on cows and pigs from our edge and urban habitats during the wet season. Additionally, we found that species and genus diversity varied when comparing canopy versus ground trap heights. We hope to utilize our data to understand Myanmar's mosquito and arboviral diversity, how mosquitoes interact with different hosts across habitats, and the role of host plasticity in arbovirus transmission.

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WHAT IS THE POTENTIAL IMPACT OF INSECTICIDE TREATED NETS IN A SEMI-ARID REGION OF NORTHERN KENYA? AN INVESTIGATION OF VECTOR POPULATIONS PRIOR TO ITN ROLL-OUT IN TURKANA, KENYA

Lucy Achieng Abel[1], Samuel Kahindi[2], David Ekai[3], Erastus Kipkosgei Kirwa[1], Rebecca Lokwang[4], Mark Erickson Amunga[1], Evans Omollo[4], Emmah C. Kimachas[1], Millicent Cherono[1], Linda Auma Maraga[1], Diana Menya[5], Andrew Obala[6], Wendy P. O'Meara[7]

[1]Academic Model Providing access to Healthcare (AMPATH), Eldoret, Kenya, [2]School of Pure and Applied Sciences, Pwani University, Kenya, Kilifi, Kenya, [3]Department of Health services and Sanitation, Turkana County, Turkana, Kenya, [4]Duke Global Inc, Nairobi, Kenya, [5]Moi UniveMoi University College of Health Sciences, School of Public Health, Eldoret, Kenya, [6]Moi University College of Health Science, School of Medicine, Eldoret, Kenya, [7]Duke Global Health Institute, Duke University, Durham, NC, United States

The ecology of malaria in northern Kenya is markedly different from that in the south. Despite its arid conditions and sparse population, transmission in the north is both intense and highly seasonal. The region faces several threats to malaria control, including identification of *Plasmodium vivax* infection and an emerging invasive vector species, *Anopheles stephensi*. Over the past five years, perceptions of malaria risk in Turkana have shifted considerably—from being viewed as a low-risk, epidemic-prone area to one of the highest transmission zones in the country. In response, Turkana County received its first mass distribution of insecticide-treated nets in late 2024. Prior to this, we established a surveillance program to understand better mosquito vector diversity, their preference for human hosts, and their role in malaria transmission. Mosquitoes were collected over one year period using Biogents Sentinel traps baited with CO_2 and synthetic human odors in both rural and peri-urban settings. Similar traps were placed indoors and outdoors within the same households for four consecutive nights. Laboratory analysis was conducted using PCR. We described mosquito density over time, species composition, host-feeding patterns (human versus animal), and the infection rate of *P. falciparum*. We found that 20% of female *An.* mosquitoes were captured outdoors, and a higher number of mosquitoes that were collected outdoors were sporozoite positive. The proportion of mosquitoes with human DNA detected was small and not significantly different between indoor and outdoor collected mosquitoes (p=0.073). Animal bloodmeal sources were roughly ten times more common. *An. arabiensis* was the most abundant vector while *An. coluzzi*, a vector commonly found in West Africa, was the second most abundant species and had the highest *P. falciparum* infection rates. A very low number of *An. stephensi* was found and none had *P. falciparum* infection nor human bloodmeal detected. These data will be useful in predicting the impact of insecticide treated nets distribution in this unique context.

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IDENTIFYING DRIVERS OF MEDICALLY IMPORTANT MOSQUITO POPULATIONS THROUGH DRONE-ACQUIRED IMAGERY ANALYSIS FOLLOWING HURRICANE BERYL IN 2024 IN HARRIS COUNTY, TEXAS

Angela A. Zieba[1], Eden Frick[2], Maya Murry[3], Kyndall Braumuller[2], Melissa Nolan[2], Courtney Standlee[4], Sarah M. Gunter[3]

[1]UTHealth School of Public Health, Houston, TX, United States, [2]University of South Carolina, Columbia, SC, United States, [3]Baylor College of Medicine, Houston, TX, United States, [4]Harris County Public Health Mosquito & Vector Control Division, Houston, TX, United States

Hurricane Beryl hit the greater Houston area in July 2024. This storm caused major rainfall and significant wind throughout the city, resulting in damage to the built environment. It occurred during peak mosquito season with active West Nile Virus transmission. There is currently limited knowledge on post-disaster drivers of medically important mosquitoes, and we believe this event provided an ideal opportunity to evaluate the impact of disaster-related environmental changes on mosquito ecology. Using drone-acquired imagery post-disaster, we investigated the correlation between types of debris and mosquito composition and abundance across the City of Houston. Each location was surveyed once weekly over four weeks post-Hurricane Beryl and then monthly for 5 months. For each drone image, trap data was summarized and graphically represented pre- and post-storm event. Drone images were evaluated to characterize built environment and storm-related debris. An analysis was conducted to determine drivers of medically important mosquito abundance. We identified non-organic debris, specifically children's pools and tires, as significant drivers of medically important mosquitoes. We believe this study represents a proof-of-concept for using remote sensing to inform community-level abatement efforts to protect public health post natural disaster.

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FROM TISSUE TO ENVIRONMENTAL DNA: PRELIMINARY EVALUATION OF A PORTABLE QPCR ASSAY FOR *AEDES AEGYPTI* SURVEILLANCE

Johanna Carolina Molina-Tabares[1], Shehu Shagari Awandu[2], Louisa Alexandra Messenger[3], Tim CD Lucas[1], Thomas Walker[4], Erin Gorsich[4]

[1]University of Leicester, Leicester, United Kingdom, [2]Jaramogi Oginga Odinga University of Science and Technology, Kisumu, Kenya, [3]University of Nevada, Las Vegas, Henderson, NV, United States, [4]University of Warwick, Warwick, United Kingdom

The WHO African Region is undergoing significant urbanization which is predicted to result in increased transmission of arboviruses transmitted by *Aedes aegypti* mosquitoes. Effective entomological surveillance remains challenging due to limitations in rapid, field-friendly diagnostic tools. Portable qPCR platforms targeting environmental DNA (eDNA) offer a promising alternative for real-time detection, especially in resource-limited areas. We firstly undertook specificity analysis of a species-specific qPCR probe assay for *Ae. aegypti* by assessing *in silico* and *in vitro* specificity against sympatric non-target African *Aedes* mosquito species. Analytical performance was compared across two qPCR platforms, including a portable genesig q16 system that can be used outside labs. Field feasibility was tested with mosquito tissue samples collected in Kisumu, Kenya, in February 2026. Additionally, we evaluated the effect of DNA quality on qPCR amplification. The assay demonstrated high specificity in both *in silico* and *in vitro* assessments, with no cross-reactivity observed with non-target African *Aedes* species. Comparable amplification performance was observed between the portable genesig q16 and a Roche LightCycler 96 qPCR platform, although variations in DNA quality impacted detection sensitivity. Field tests confirmed that the genesig q16 platform can reliably detect *Ae. aegypti* from tissue samples and testing eDNA collected from field water samples is ongoing. These findings highlight the potential of portable qPCR as an effective tool for *Ae. aegypti* surveillance in the WHO

African Region. Ultimately, this work contributes to scalable surveillance strategies that can inform *Ae. aegypti* distribution mapping within the WHO African Region to mitigate future arboviral outbreaks

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COMPARISON OF *AEDES AEGYPTI* AND *AE. ALBOPICTUS* LARVAL HABITATS IN TWO DENGUE-ENDEMIC AREAS OF COSTA RICA WHERE BOTH SPECIES COEXIST

Kota Osawa-Pivovarov[1], Betzabé Alfaro-Salazar[1], Talya Shragai[2], Diana Rojas-Araya[1], Caroline Glidden[2], Luis E. Chaves-González[1], Erin Mordecai[2], Adriana Troyo[1]

[1]Universidad de Costa Rica, San Jose, Costa Rica, [2]Stanford University, Stanford, CA, United States

Dengue vectors *Aedes aegypti* and *Aedes albopictus* are present throughout Costa Rica and frequently coexist in rural areas. *Aedes albopictus* has expanded in the country over the past 20 years, but its interactions with *Ae. aegypti*, population dynamics, and larval ecology is not well known. This study aimed to describe and compare *Ae. aegypti* and *Ae. albopictus* larval habitats associated with forest, agriculture, and residential environments in two dengue-endemic rural regions of Costa Rica: Quepos-Parrita (QP), Puntarenas province, and Sarapiqui (SA), Heredia province. At each site, larval surveys were carried out in 50 locations (house, field, forest, etc.) during the 2025 wet season. The "location" (LI) and "Breteau location" (BI) indices were calculated for each *Aedes* species. In QP, 77/115 (67.0%) larval habitats were positive for *Aedes* spp.: 4 (3.5%) for *Ae. aegypti* only, 61 (53.0%) for *Ae. albopictus* only, and 12 (10.4%) for both species. In SA, 56/113 (49.6%) were positive for *Aedes* spp.: 15 (13.3%) for *Ae. aegypti* only, 29 (25.7%) for *Ae. albopictus* only, and 12 (10.6%) for both. LI and BI were higher for *Ae. albopictus* than for *Ae. aegypti* at both sites. However, QP had higher *Ae. albopictus* indices (LI=66; BI=145) than SA (LI=35.3; BI=82), while *Ae. aegypti* indices were higher in SA (LI=23; BI=54) than in QP (LI=20; BI=32). The most common *Aedes* spp. habitat types in QP were tires, buckets, and other miscellaneous containers (e.g., trash), while in SA they were leaf axils, coconuts, plastic tarps, and miscellaneous containers. Although habitat positivity in forest and agriculture environments was higher for *Ae. albopictus*, *Ae. aegypti* habitats were more common in residential areas. Other larvae collected included species of the genera *Limatus, Culex, Uranotaenia, Isostomyia, Wyeomyia, Shannoniana, Trichoprosopon, Haemagogus,* and *Toxorhynchites*. Results show that *Ae. aegypti* and *Ae. albopictus* are abundant and commonly use the same containers in these areas. Future research will determine if *Ae. albopictus* outcompetes *Ae. aegypti*, or if they will continue to coexist by occupying specific niches.

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INSECTICIDE SUSCEPTIBILITY PROFILE AND METABOLIC RESISTANCE MECHANISMS OF *ANOPHELES MELAS* IN MBINE COLY, SENEGAL

Sy Ousmane[1], Mohamed Abderemane Nourdine[1], Pape Cheikh Sarr[1], Assane Ndiaye[1], Lassana Konaté[1], Ousmane Faye[1], Oumar Gaye[1], Ellen Dotson[2], El Hadji Amadou Niang[1]

[1]University Cheikh Anta Diop, Dakar, Senegal, [2]Centers for Disease Control and Prevention, Atlanta, GA, United States

In Senegal, the persistence of residual malaria transmission in coastal areas is closely linked to the presence of the secondary vector *Anopheles melas*. This study aims to characterize the insecticide susceptibility profile and resistance mechanisms of a wild population collected in Mbine Coly (Mbour district). Larvae were sampled in September 2024 from brackish water habitats (salinity 10-18 g/l). Following molecular identification (100% *An. melas*), WHO bioassays (1X, 5X, and 10X diagnostic doses) were conducted on F2 to F8 generation. The involvement of metabolic enzymes was evaluated through synergy tests using pre-exposure to piperonyl butoxide (PBO). Confirmed resistance to alpha-cypermethrin (81.7% mortality) and suspected resistance to deltamethrin (91.3%) and permethrin (94.3%) were observed. Statistical analyses showed that PBO pre-exposure significantly restored full susceptibility (100%) for deltamethrin (p;=0.003) and permethrin (p;=0.028), confirming the major role of P450 enzymes. For alpha-cypermethrin, resistance was of moderate-to-high intensity, persisting at the 5X dose (95.5%, p;<0.001). Conversely, full susceptibility (100%) was recorded for pirimiphos-methyl and bendiocarb. In conclusion, Pyrethroid resistance in *An. melas* from Mbour is primarily metabolically mediated. These findings suggest that the deployment of next-generation bed nets (PBO-LLINs) and insecticide rotation (organophosphates/carbamates) are priority strategies for the National Malaria Control Program (NMCP) to counter residual transmission in this area.

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OVIPOSITION PREFERENCE OF FEMALE *ANOPHELES ARABIENSIS* TO HABITATS WITH INTRASPECIES AND INTERSPECIES MOSQUITO LARVAL COMPETITORS AND THEIR EFFECT ON LARVAL DEVELOPMENT

Hudson Onen[1], Emmanuel W. Kaindoa[2], Perpetra Akite[3], Jonathan K. Kayondo[1], Martha A. Kaddumukasa[4], Anne M. Akol[3], Frederic Tripet[5]

[1]Department of Entomology, Uganda Virus Research Institute, Kampala, Uganda, [2]Environmental Health and Ecological Science Department, Ifakara Health Institute, Dae es salaam, United Republic of Tanzania, [3]Department of Zoology, Entomology and Fisheries Sciences, College of Natural Sciences, School of Biosciences, Makerere University, Kampala, Uganda, [4]Faculty of Science, Biological Sciences, Kyambogo University, Kampala, Uganda, [5]Swiss Tropical and Public Health Institute, Allschwil, Switzerland

Some species of the *Anopheles gambiae* complex are major malaria vector. Females of these species oviposit in aquatic habitats used by other mosquitoes. It is unclear if gravid *An. arabiensis* avoid laying eggs in habitats containing intraspecies and interspecies larval competitors and if they affects larval development. 72 hrs prior to the experiment, 1st instar of *An. gambiae* s.s., *An. arabiensis* and *Cx. quinquefasciatus* were allocated to 12 randomised habitats in a semi-field. Eighty gravid *An. arabiensis* were released in each compartment of the semi-field to lay eggs. Eggs from each habitat were collected after 24 hrs, counted and raised to 3rd instars for sibsip analyses or kept in the habitats to record their development. Larval progeny from one compartment were analysed at 9 microsatellite loci to identify siblings via a maximum likelihood approach to reconstruct egg-laying strategies of each female. Presence and kind of larval species in the habitat significantly influenced habitat selection by gravid *An. arabiensis*. Significantly more eggs were laid in habitats without larvae, followed by those with *An. arabiensis*, *An. gambiae* s.s., and *Cx. quinquefasciatus* larvae. Larval development followed a similar pattern, with more larvae developing to 4th instar and pupae in competitor free habitats, followed by those with *An. arabiensis*, *An. gambiae* s.s. and *Cx. quinquefasciatus* larvae. The genetic sibship analyses revealed that most females did not skip oviposit and preferentially laid eggs in competitor free habitats followed by those containing *An. arabiensis*, *Cx. quinquefasciatus* and *An. gambiae* s.s. larvae. The few females that skip oviposited, preferentially did across habitats without competitors followed by *An. gambiae* s.s. but not *An. arabiensis* and *Cx. quinquefasciatus* larvae.The study has shown that the presence of intraspecies and interspecies larval competitors in aquatic habitats significantly affect the oviposition site selection behaviour of female *An. arabiensis*, which in turn impacts the developmental success of their progeny.

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DEVELOPMENT AND PRELIMINARY EVALUATION OF VECTORPAL: A MORPHOLOGICAL IDENTIFICATION TOOL FOR ANOPHELES MOSQUITOES ROBUST TO UNCERTAIN AND INCOMPLETE OBSERVATIONS

Ana Paula Pérez-Aguirre[1], Sai L.R. Devireddy[1], Sunny Patel[1], Kigongo Siriman[2], Marina Rincon Torroella[1], Aryaman Shodhan[1], Luis R. Soenksen[1], Neil F. Lobo[3], Soumyadipta Acharya[1]

[1]Johns Hopkins University, Center for Bioengineering Innovation & Design, Baltimore, MD, United States, [2]Vector Control Division, Kampala, Uganda, [3]University of Notre Dame, South Bend, IN, United States

Accurate identification of malaria vectors remains a bottleneck for targeted control in endemic regions. While molecular identification is the gold standard, its cost is not scalable for low-resource settings, which rely on time-consuming and error-prone morphological identification, which can take up to 30 minutes per specimen, and can be affected by collection methods that damage the mosquito. We developed VectorPal, a digital tool for morphological identification that can handle damaged specimens by automating navigation of dichotomous keys, implementing rule-based filtering and integrating a Large Language Model (LLM) for optional natural language input. Based on Coetzee's Afrotropical Anopheles key (2020), we developed a database of 151 entries representing mosquito species and morphological variants. Each of 128 features was encoded as 0, 1, or absent when not present in the key, and each entry was reviewed against the key by two study team members. We then implemented a rule-based filtering system comparing the features of the observed specimen to the database, deterministically eliminating discrepant species. The remaining candidates are ranked using a discrete Bayesian approach. The rule-based filtering system showed complete fidelity to the dichotomous key, consistently matching the ground truth species when given the full set of features. When provided with incomplete input to simulate cases with missing or ambiguous features, the system outputted a list of possible candidates, which included the ground truth label in all cases. The number of candidate species decreased along with an increasing number of features provided, from an average of 61 candidates with 1 feature to fewer than 5 when given five features. For natural language input, the top-performing LLM had a 91% feature-wise accuracy when mapping descriptions to features. This shows potential for VectorPal to enable accurate and scalable species identification in low-resource settings where molecular identification is unfeasible, laying the groundwork for adaptations for quality control, entomologist training, and accelerated field identification.

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SURVEILLANCE OF HUMAN AND *ANOPHELES* SPECIES SPECIFIC BEHAVIORS TO UNDERSTAND GAPS IN PROTECTION AGAINST MALARIA TRANSMISSION AT SENTINEL SITES IN UGANDA

Marvious Kiwanuka[1], Alex K. Musiime[2], Max McClure[3], Perpetra Akite[1], Maxwell Kilama[2], Daniel Basangwa[4], Edward Thomsen[5], Catherine Maiteki-Sebuguzi[6], Joaniter Nankabirwa[7], Moses R. Kamya[1], Grant Dorsey[8], Neil Lobo[9], Paul Joseph Krezanoski[10]

[1]Makerere University, Kampala, Uganda, [2]Kampala, Uganda, [3]UCSF, San Francisco, CA, United States, [4]Infectious Diseases Research Collaboration (IDRC), 2C Nakasero, PO Box 7475, Uganda., Kampala, Uganda, [5]Malaria Elimination Initiative (MEI), Institute for Global Health Sciences, University of California, San Francisco (UCSF), Mission Hall, San Francisco, CA 94143, San Francisco, CA, United States, [6]Ministry of Health, Kampala, Uganda, [7]Makerere Univ Kampala, Kampala, Uganda, [8]Univ of California San Francisco, San Francisco, CA, United States, [9]Univ of Notre Dame, South Bend, IN, United States, [10]University of San Francisco, California (UCSF), San Francisco, CA, United States

Malaria control in Uganda relies on long-lasting insecticide-treated nets (LLINs) and indoor residual spraying (IRS), yet persistent transmission remains due to rising insecticide resistance, shifts in vector behaviors, and heterogeneous LLIN use. This study aims to identify gaps in protection that sustain residual transmission across diverse ecological and intervention settings in Uganda. As part of the ongoing Human and Vector Interaction (HAVI) study, we are conducting integrated human behavioral and entomological surveillance at sentinel sites every 4 weeks across 17 medium- and high- transmission districts in Uganda. The goal is to quantify human-vector contact, evaluate the effectiveness of current interventions, and characterize species-specific biting patterns of *Anopheles* mosquitoes. Human landing catches (HLCs) are performed in six households per site, both indoors and outdoors, alongside structured observations of human behavior to capture hourly patterns of location, LLIN use, and exposure to infective bites.Female *Anopheles* mosquitoes collected via HLCs by hour and location of collection are assigned standardized identifiers, morphologically identified to species, assessed for abdominal status, and linked to detailed case report forms. *Plasmodium falciparum* sporozoite infection is determined by ELISA. A subset of samples will undergo species confirmation by PCR, with remaining material archived in Uganda for future analyses. We anticipate that approximately 5 months of the data will available for analysis by the time of the conference. We plan to use generalized additive mixed effects models to (1) estimate species-specific, human behaviour-adjusted infectious biting rates; (2) quantifying the proportion of exposure occurring indoors vs outdoors, and while awake vs asleep with/without LLINs; (3) identifying ecological and behavioural correlates of high residual exposure. Based on the outcome of this analysis will define spatial and temporal transmission patterns, and provide evidence to optimize the selection and targeting of malaria vector control strategies in Uganda.

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LINKING ENVIRONMENTAL HETEROGENEITY TO VECTOR COMPOSITION AND PLASMODIUM TRANSMISSION ACROSS ECOZONES IN BURKINA FASO: INSIGHTS FROM THE PIVOTAL INTERNATIONAL CENTERS OF EXCELLENCE FOR MALARIA RESEARCH (PIVOTAL ICEMR),

Elizabeth Hemming-Schroeder[1], Salomon Zidouemba[2], Patindé Didier Alexandre KABORE[3], Frederic Wendlamita Nikiema[4], Amy Kristine Bei[5], A. Fabrice Some[6], Sunil Parikh[5], Brian D. Foy[1]

[1]Colorado State University, Fort Collins, CO, United States, [2]Institut de Recherche en Science de Santé, Ouagadougou, Burkina Faso, [3]Institut de Recherche en Sciences de la Santé (IRSS), Bobo-Dioulasso, Burkina Faso, [4]Instiut de Recherche en Science de la Sante de Bobo Dioulasso, Bobo Dioulasso, Burkina Faso, [5]Yale School of Public Health, New Haven, CT, United States, [6]IRSS-DRO, Hauts-Bassins, Burkina Faso

Malaria transmission is shaped by interacting biological, environmental, and human factors across spatial and temporal scales. Environmental conditions structure ecological niches that differentially support mosquito vector species, leading to heterogeneity in vector distribution. Because vector species differ in ecology, behavior, and competence for transmitting Plasmodium species, environmental heterogeneity influences parasite distribution and transmission intensity. While *Plamodium falciparum* remains the predominant cause of malaria in much of Africa, "minor" species such as P. malariae and P. ovale are increasingly recognized as public health concerns. This study was motivated by the repeated detection of non-falciparum parasites and the hypothesis that environmentally structured variation in vector species composition may explain their spatiotemporal distribution. This ongoing study, conducted within the Burkina Faso International Centers of Excellence for Malaria Research (PIVOTAL ICEMR), aims to quantify how environmental drivers shape vector dynamics and malaria transmission across three ecozones. Longitudinal entomological data were collected from four sites per ecozone (12 total), comprising over 3,000 blood-fed Anopheles mosquitoes sampled across four seasonal timepoints over one year. Data were linked to environmental covariates derived from remote sensing, including landscape metrics (NDVI, NDMI, NDBI, land cover) from Sentinel-2 satellite mission imagery across multiple spatial buffers and lag periods. Mosquito outcomes were analyzed using generalized linear mixed models accounting for spatial clustering and overdispersed counts. Vector counts were modeled by species, and infection status was evaluated for multiple Plasmodium species. Preliminary results indicate that vegetation structure and surface moisture are key

predictors of vector presence and density, with effects varying across vector species. Ongoing analyses integrate entomological and parasitological data to assess how vector community variation influences infection rates and transmission patterns.

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MOLECULAR EPIDEMIOLOGY AND GENOTYPE DIVERSITY OF HUMAN ENTEROVIRUSES IN PEDIATRIC ACUTE UNDIFFERENTIATED FEBRILE ILLNESS IN BRAZIL

Leonardo Cecílio Rocha[1], Vanessa Cristina Martins Silva[1], Victoria Bernardi Ciconi[2], Cecilia Artico Banho[2], Beatriz Carvalho Marques[2], Livia Sacchetto[2], Laís Sampaio Azevedo[1], Adriana Parise[1], Cassia Fernanda Estofolete[2], Tatiana Sakomura[2], Mauricio Lacerda Nogueira[2], Adriana Luchs[1]

[1]Adolfo Lutz Institute, São Paulo, Brazil, [2]Faculty of Medicine of São José do Rio Preto, São José do Rio Preto, Brazil

Human Enteroviruses (HEV) are underdiagnosed causes of acute undifferentiated febrile illness (AUFI) in children due to their nonspecific presentation. This study evaluated the frequency and molecular diversity of HEV in patients ≤14 years with AUFI treated at a tertiary hospital in São José do Rio Preto, Brazil, aiming to characterize circulating genotypes and infer dissemination patterns. A total of 405 oropharyngeal swabs collected between 2021 and 2024 were analyzed by in-house RT-qPCR. Positive samples underwent conventional RT-PCR targeting VP1, Sanger sequencing, phylogenetic analysis and isolation in RD cells to assess infectivity. HEV was detected in 7.2% (29/405) of cases, with ages ranging from 0 to 10 years (mean 3.3; median 3), with higher frequency in children aged 2-4 years (62.1%; $p < 0.05$). Cytopathic effect was observed in 79.3% (23/29) and genotyping was successful in 37.9% (11/29). Coxsackievirus (CV)-B5 was identified in five samples, while CV-B1, CV-B2, CV-A21, CV-A5, CV-A8 and Echovirus (E)-3 were each detected in one sample. CV-B1/FAMERP-93/2022 showed high identity (96-97.2%) with Chinese strains, suggesting Asian origin. CV-B2/FAMERP-158/2023 was genetically distant from Brazilian strains (77.6-79.7%), indicating underexplored diversity. CV-B5 strains clustered with Brazilian strains (98.4-100%), reflecting endemic circulation. E-3/FAMERP-96/2022 was closer to European strains (97.1-97.9%) than Brazilian ones (88.0-90.9%), indicating recent introduction. CV-A5/FAMERP-307/2023 showed high identity with Russia, France and USA strains (96.9-98%) but lower similarity to Brazilian strains (80.3-88%). CV-A8/FAMERP-400/2024 was related to a Japanese strain (96-97%). CV-A21/FAMERP-198/2023 showed high identity with global strains (92.7-98.8%), especially a 2023 Russian isolate (98.8%). This study demonstrated circulation of multiple HEV genotypes, including the first report of CV-A21 in Brazil. Oropharyngeal swabs proved a practical and effective tool for HEV detection and surveillance in AUFI

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SEROPREVALENCE AND ASSOCIATED RISK FACTORS OF RIFT VALLEY FEVER IN HUMANS FROM THREE ECOLOGICAL ZONES OF SENEGAL

Elisabeth Thérèse FAYE[1], Samba Niang Sagne[1], Mareme Thiam[1], Noé Ochida[2], Ousseynou Sene[1], Sokhna Mayemouna Diop[1], Ndeye Sakha Bob[1], Moussa Dia[1], Moussa Moïse Diagne[1], Oumar Faye[1], Ndongo Dia[1], Diawo Diallo[1], Etienne Simon- loriere[2], Anavaj Sakuntabhai[2], Cheikh Talla[1], Simon Cauchemez[2], Cheikh Loucoubar[1], Malick Fall[3], Mamadou Aliou Barry[1], Gamou Fall[1]

[1]Institut Pasteur de Dakar, Dakar, Senegal, [2]Institut Pasteur de Paris, Paris, France, [3]Animal Biology Department, Faculty of Sciences and Techniques, Cheikh Anta Diop University, Dakar, Senegal

Rift Valley fever (RVF) is an emerging arboviral zoonosis with significant public health impact in Africa. In Senegal, sporadic human cases have been reported since 1987, with a major outbreak in September 2025. However, population-level data on human exposure remain limited. This study aimed to estimate RVFV seroprevalence, identify associated risk factors, and reconstruct transmission dynamics in Senegal. A cross-sectional seroepidemiological study was conducted between 2022 and 2024 in three ecologically distinct sites: Podor, Linguere, and Kedougou. Healthy individuals with varying levels of animal exposure (daily, weekly, occasional, or none) were enrolled. Serum samples were tested for anti-RVFV IgM and IgG using ELISA, with confirmation by PRNT. Multivariable logistic regression identified risk factors associated with IgG seropositivity, and serocatalytic modeling was applied in Podor. Among 861 participants, IgG seroprevalence was 27.7% in Podor, 4.2% in Linguere, and 0.7% in Kedougou, resulting in an overall seroprevalence of 10.6%. Seroprevalence was higher in men and increased with age. Individuals with frequent animal contact had higher exposure, and exposure was more common during the rainy season. In multivariable analysis, only age remained significantly associated with seropositivity. Serocatalytic modelling in Podor identified three distinct periods characterized by increased forces of infection. These findings highlight heterogeneous RVFV exposure and long-term virus circulation in Senegal, emphasizing the need for strengthened integrated surveillance and targeted prevention strategies.

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RECOMBINANT PROTEIN-BASED VACCINES PROTECT AGAINST NEUROINVASIVE LA CROSSE VIRUS CHALLENGE

Pallavi Rai

Virginia Tech, Blacksburg, VA, United States

La Crosse Virus (LACV) is the leading cause of pediatric viral encephalitis in North America, particularly in the Midwest and Appalachian areas, with no licensed vaccines available. Initial symptoms include fever, headaches, nausea, vomiting and fatigue with severe cases ending in encephalitis, seizures and occasionally, coma and cognitive changes. Seroprevalence studies suggest underreported infections, underscoring the need for improved preventive strategies in endemic areas. To address the need for a LACV vaccine, we evaluated the immunogenicity and efficacy of recombinant protein-based vaccine candidates against LACV. Mice were immunized with recombinant glycoproteins (GcS or GnGcF) formulated with or without adjuvants (IVAX1 or DOTMA) and subsequently challenged with a contemporary LACV strain, TX09. Sham vaccinated mice challenged with LACV exhibited significant weight loss and 100% mortality by 8 days-post-challenge (DPC). Immunization with GcS or GnGcF alone presented disease similar to sham in terms of weight loss, mortality and high brain titers, thus conferring no protection. There was no evidence of weight loss or brain viral titers in either adjuvanted candidates, GcS and GnGcF with IVAX1 and DOTMA. In terms of survival, performance with IVAX1 adjuvanted vaccinated mice were completely protected from mortality while DOTMA adjuvanted mice showed 90% survival. Neutralizing antibody responses measured by plaque reduction neutralization tests (PRNTs) were significantly increased with both adjuvants. At 7 and 21 days-post-boost (DPB), GcS+IVAX1 induced highest neutralizing antibody titers (3.8 $\log_{10}$ $PRNT_{50}$; SD:0.25), followed by GcS+DOTMA (3.2 $\log_{10}$ $PRNT_{50}$; SD:0.29). However, GnGcF-based vaccines showed moderate neutralizing antibody responses up to 21 DPB. These studies suggest that IVAX1-adjuvanted recombinant protein vaccine candidates elicited robust neutralizing antibody responses and complete protection from LACV lethal challenges. Altogether, our data supports these as promising candidates for LACV and warrants further pre-clinical evaluation.

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VIROME OF BATS COLLECTED IN THE CAVES OF MOUNT ELGON, KENYA

Sarah Waswa, Samuel Kariuki

University of Eldoret, Eldoret, Kenya

Bats account for ~25% mammalian biomass and possess immune adaptations enabling them to carry diverse viruses and potentially transmit them to humans/animals. We used metagenomic sequencing to characterize bat viromes from caves in the Mt. Elgon region, western Kenya. Eighty-six bats were sampled in March and September 2024 at three cave sites in Bungoma County. Oral and rectal swabs were

taken, total nucleic acids extracted, reverse-transcribed, and libraries enriched with VirCapSeq-VERT probes. Libraries were sequenced on an Illumina NextSeq 2000. Reads were quality-filtered and analyzed for host species and viral composition. Molecular identification found seven bat species; *Coleura afra* dominated (79.01%). Other species: *Miniopterus inflatus* (6.17%), *Nycteris thebaica* (3.70%), *Hipposideros ruber* (3.70%), *Rhinolophus cf. eloquens* (3.70%), *Lissonycteris angolensis* (2.47%), *M. natalensis* (1.23%). Metagenomics detected 20 viral species across 15 viral families (12 mammalian, 2 insect, 1 plant/fungal). In 71 bats that were sequenced, major detections were for Retroviridae (77.46%, predominantly gammaretroviruses at 73.24%), Parvoviridae (29.58%, mainly Densovirus), Genomoviridae (21.13%, Gemykibivirus), Paramyxoviridae (12.68%, Bat orthoparamyxovirus), Astroviridae (7.04%, Bat astrovirus), Orthocoronavirinae (7.04%, bat coronaviruses), Nodaviridae (5.63%, Nodavirus), and Sedoreoviridae (4.23%, including Rotavirus groups H, C, and D). Coleura afra was the dominant species sampled. The bat virome was dominated by gammaretroviruses. Detection of rotavirus H, C, and D underscores bats' roles in shaping viral diversity and potential spillover risk, supporting enhanced One Health surveillance and genomic monitoring.

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HOST COMPETENCE, IMMUNE RESPONSES, AND TOLERANCE STRATEGIES SHAPE WEST NILE VIRUS AMPLIFICATION IN NEOTROPICAL AVIAN HOSTS

Octavio Giayetto[1], Agustin I. E. Quaglia[2], Gabriel Barco[3], Franco N. Nazar[4], Adrian Diaz[5]

[1]National Scientific and Technical Research Council, Córdoba, Argentina, [2]National Scientific and Technical Research Council, San Luis, Argentina, [3]Laboratorio de Arbovirus- Instituto de Virología, Córdoba, X, Argentina, [4]Analytical Microsystems & Materials for Lab-on-a-Chip (AMMa-LOAC) Group, Microfluidics Cluster UPV/EHU, Analytical Chemistry Department, University of the Basque Country, Lejona 48940, Spain., Vitoria-Gasteiz, Spain, [5]Instituto de Virología, Cordoba, Argentina

Generalist arboviruses such as West Nile virus (WNV) are maintained through complex transmission networks involving multiple avian hosts and mosquito vectors. These dynamics are strongly influenced by heterogeneity in host competence, reflecting each species' contribution to viral maintenance. We evaluated host competence in four Neotropical avian species (*Agelaioides badius*, *Molothrus bonariensis*, *Columbina picui*, and *Zenaida auriculata*) by integrating viral amplification with immunological and physiological responses during experimental infection. Individuals were experimentally infected and monitored over time. Viral amplification, constitutive humoral and cellular immunity, and body mass changes were quantified, and host competence indices were estimated. Viral replication dynamics differed significantly among species over time (GAMM, $p < 0.001$). *M. bonariensis* and *C. picui* showed the highest viral amplification (up to 4.5 $\log_{10}$ PFU/ml), whereas *A. badius* exhibited the lowest peak viremia (3.3 $\log_{10}$ PFU/ml), consistent with higher resistance. Host competence indices reflected these patterns: 0.71 (95% CI: 0.40–1.02) for *M. bonariensis*, 0.59 (0.30–0.89) for *C. picui*, 0.49 (0.15–0.82) for *Z. auriculata*, and 0.26 (0.00–0.47) for A. badius. Monocyte proportions (GAMM, $p = 0.005$) and natural antibody titers ($p = 0.041$) varied among species. Body mass loss showed a species-specific temporal response (GLMM, $p < 0.001$), supporting interspecific differences in tolerance inferred from the relationship between viremia and mass loss. By integrating viral amplification with host responses, our findings clarify how species-specific resistance and tolerance profiles shape avian contributions to WNV transmission. These results provide insight into the eco-epidemiological processes underlying WNV maintenance at its southernmost distribution limit.

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FIRST LABORATORY-CONFIRMED FATAL DENGUE VIRUS INFECTION AND ITS IMPLICATIONS FOR NATIONAL ARBOVIRAL SURVEILLANCE IN LIBERIA

Bode I. Shobayo[1], Kalilu S. Donzo[1], Emmanuel Dwalu[1], Ralph W. Jetoh[1], Fahn M. Tarweh[1], Julius S.M. Gilayeneh[1], Sia W. Camanor[1], Methodius George[2], Chukwuma D. Umeokonkwo[3], Stephen B. Kennedy[4]

[1]National Public Health Institute of Liberia, Monrovia, Liberia, [2]Ministry of Health, Republic of Liberia, Monrovia, Liberia, [3]African Field Epidemiology Network (AFENET), Kampa, Uganda, [4]PREVAIL, Monrovia, Liberia

Dengue Virus (DENV) is an emerging public health threat in Africa, where its impact is underestimated due to non-specific clinical manifestations and restricted diagnostic capabilities. In Liberia, laboratory-confirmed cases of DENV were initially recorded in an urban febrile surveillance study (2018-2020), yet fatal outcomes had not been previously reported. This paper reports the first laboratory-confirmed fatal dengue case and its implications for national surveillance and policy in Liberia. On February 2, 2026, a 38-year-old Liberian male arrived at J.J. Dossen Hospital in Maryland County, Southern Liberia, with two days history of high fever, severe headache, myalgia, weakness, and abdominal discomfort but no recent history of travel. The first laboratory investigation using rapid test and microscopy ruled out malaria. Molecular detection performed at the National Public Health Reference Laboratory was negative for Lassa, Ebola, and Marburg. Despite management with supportive care, his condition deteriorated clinically upon re-admission, and died on February 6, 2026. Serological testing performed in Ghana detected DENV specific-IgG and NS1 protein positive, suggestive of prior or recent exposure to Dengue fever, prompting further investigations. Blood sample was referred to the Institut Pasteur de Dakar, Senegal, where differential RT-PCR testing was conducted for diverse array of arboviruses, viral hemorrhagic fevers, and viral-specific IgM ELISA, confirmed positive for DENV RNA by RT-PCR and negative for other viruses. Dengue-specific IgM was negative, consistent with an acute primary infection during the early viremic phase, prior to IgM seroconversion. This is the first molecular confirmation of fatal dengue in Liberia. It highlights the potential for severe outcomes and indicates local transmission in a rural, cross-bordered region of the country. There is critical need for swift implementation of integrated arboviral surveillance in both urban and rural settings, better diagnostic capabilities, and possibly a nationwide sero-epidemiological study to estimate the actual burden of dengue in the country.

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EVALUATION OF CONCENTRATION AND EXTRACTION METHODS FOR DETECTING MULTIPLE PATHOGENS FROM WASTEWATER IN DHAKA, BANGLADESH

Sabrina Karim Resha

International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Dhaka, Bangladesh

Wastewater-based surveillance (WBS) is a powerful tool for monitoring infectious diseases in resource-limited settings where clinical surveillance is limited. Most WBS protocols detect only a few pathogens, restricting multi-pathogen monitoring. Developing robust methods to concentrate and detect multiple pathogens from a single sample is critical. We evaluated five concentration and extraction workflows to identify an optimal approach for broad-spectrum pathogen detection. Wastewater samples (n=10) were collected in Dhaka, Bangladesh and aliquoted into various volumes (10 mL, 35 mL, 160 mL and 40 mL) for downstream processing. For Methods A (10 mL), B (35 mL), and D (160 mL), Nanotrap® Microbiome A Particles were used for concentration, followed by Nanotrap® PRIME Kit based extraction. Method C (160 mL) involved concentration using Nanotrap® Microbiome A particles and extraction was subsequently performed with the MagMAX™ Wastewater Ultra Nucleic Acid Isolation Kit. In Method E (40 mL), polyethylene glycol-based concentration was conducted, followed by extraction with the MagMAX™ Wastewater Ultra Nucleic Acid Isolation

Kit. Nucleic acids were tested using a custom TaqMan Array Card (TAC) for 48 pathogen targets (22 bacterial and 24 viral targets, 2 protozoa), 1 manufacturing control and 4 extrinsic control targets. Detection efficiency varied across methods. Method C showed the highest viral (median 44%, range 39-50%) and bacterial (median 68%, range 59-77%) detection across samples compared to the other methods. Comparing with other methods, Method C yielded higher gene copies for most viral (rotavirus, norovirus, sapovirus, adenovirus) and bacterial targets (*Shigella spp., S.flexneri 6*, enterotoxigenic and enteroaggregative *Escherichia coli*) (Wilcoxon signed rank test, $p<0.05$). Overall, Method C (concentration with Nanotrap® Microbiome A coupled with MagMAX™ Wastewater Ultra Nucleic Acid Isolation Kit extraction) demonstrated superior multi-pathogen detection, enabling broader, more sensitive recovery from a single sample. This workflow offers a scalable approach to improve WBS for monitoring public health.

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ENABLING VACCINE TRANSLATION FOR EMERGING VIRAL AND PARASITIC TROPICAL DISEASES

António Roldão, Ricardo Correia, Margarida Q. Rodrigues
Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal

The development of vaccines for neglected and emerging tropical diseases is often constrained by antigen discovery and manufacturing readiness. Here, we present a cross-disease approach that places bioprocess development, scalability, GMP compliance, and structured technology transfer at the core of advancing subunit vaccine candidates targeting priority pathogens. Within international consortia, we supported the development of vaccine candidates for Chagas disease and malaria by establishing scalable, GMP-compliant processes that advanced to GMP manufacturing. For Chagas disease, Traspain chimeric protein was produced in *E.coli* at 50L scale in bioreactors, refolded, concentrated by TFF, purified via IMAC and SEC, and formulated in an arginine-based buffer with a STING-agonist adjuvant. For malaria, PfRipr5 protein was expressed in insect High Five cells at 50L scale in bioreactors, purified using IMAC and SEC, concentrated by TFF, and formulated with a TLR7-agonist adjuvant. In both cases, GMP-grade material supported immunogenicity and GLP toxicology studies in representative animal models, with proven safe profile and potent immunogenicity. In house, we developed a vaccine candidate for Rift Valley fever based on ferritin nanoparticles mediated display of Gn antigen, using either genetic fusion or tyrosinase-mediated bioconjugation. In the geneticfusion approach, ferritin-Gn was produced in insect cells, whereas in the bioconjugation approach ferritin was produced in *E.coli* and Gn in insect cells. Resulting nanoparticles were purified via IMAC and SEC, characterized using biophysical methods (e.g., HPLC, DLS, EM, mass photometry), and evaluated in vitro using THP-1-derived dendritic cells, where they showed enhanced internalization, maturation marker upregulation, and proinflammatory cytokine secretion compared to soluble Gn antigen, supporting their progression toward in vivo immunogenicity studies in murine models. Together, these studies underscore the importance of manufacturing-focused innovation in advancing next-generation vaccines for tropical and emerging diseases.

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ASSESSMENT OF INTEGRATIVE GLYCO-IMMUNOLOGY SIGNATURES AS A CANDIDATE FOR PREDICTOR OF DENGUE SEVERITY

Vishwa Deepak Tiwari[1], Santanu Singh[2], Thakur Shubh Narayan Rai[1], Deepak Kumar[1], Sumit Kumar Singh[2], Gopal Nath[1], Jaya Chakravarty[1]
[1]Institute of Medical Sciences, Banaras Hindu University, Varanasi, India, [2]Indian Institute of Technology, Banaras Hindu University, Varanasi, India

Dengue is a serious global health issue, ranging from mild Dengue Fever (DF) to Dengue Fever with Warning Signs (DFWS) and Severe Dengue (SD) as per the 2009 WHO guidelines. Early identification is crucial for clinical management of patients at the risk of disease severity. This study explores candidacy of Th1/Th2/Th17 cytokine profiles and IgG N-Glycans as potential severity predictors in dengue patients. Patient samples (N=151) were characterized positive for dengue by NS-1/IgM ELISA (InBios). Serum albumin, alanine and aspartate aminotransferase (ALT, AST) of patients were also evaluated. Confirmed patients (n=64) having no co-infection, were selected and categorised into DF (n=12), DFWS (n=26), and SD (n=26), alongwith Healthy Control (n=12). Th1/Th2/Th17 cytokines were quantified in blood plasma of categorised patients using CBA analysis. N-glycan analysis was performed by HILIC based UPLC-FLD system. It involves IgG purification, N-glycan release, and fluorescent labelling. The dextran ladder was used as calibration standard. Statistical analysis employed Shapiro-Wilk test for distribution assessment, followed by ANOVA/Tukey's post-hoc. IL-17 was significantly elevated in DFWS and SD compared to HC, with no significant difference in DF. IFN-γ, TNF-α, and IL-4 levels were significantly higher in DFWS compared to DF and significantly lower in SD compared to DFWS. IL-10 and IL-6 were significantly elevated in SD compared to DF and DFWS. IL-2 was elevated across all dengue groups compared to HC, with progressive elevation from DF to DFWS to SD. Low serum albumin and high ALT and AST were significantly associated with SD when compared with DFWS and DF. Alterations in glycosylation, sialylation, and fucosylation correlated with inflammatory mediators, forming a glyco-inflammatory axis. A composite Glyco-inflammatory Index captured these patterns and enabled patient stratification. Unsupervised clustering revealed hidden molecular heterogeneity in early-stage dengue, highlighting predictive potential.

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SEROTYPE-SPECIFIC ANTIBODY PROFILING REVEALS ORIGINAL ANTIGENIC SIN IN SECONDARY DENGUE VIRUS (DENV) INFECTIONS AND ENABLES RECONSTRUCTION OF INFECTION HISTORY

Christa Ehmen[1], Rutineia Ferraz[1], Jörg Blessmann[1], Yvonne Winkelmann[1], Latdamone Keoviengkhone[2], Vatsana Sopraseuth[2], Simone Kann[3], Petra Emmerich[1], Herbert Schmitz[1], **Christina Deschermeier**[4]
[1]Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany, [2]Savannakhet Provincial Hospital, Savannakhet, Lao People's Democratic Republic, [3]Bundeswehr Central Hospital, Koblenz, Germany, [4]Panadea Diagnostics GmbH, Hamburg, Germany

Understanding how prior dengue virus (DENV) exposures shape immune responses is critical for interpreting disease risk and vaccine performance. However, serological determination of previously encountered DENV serotypes is hampered by antigenic cross-reactivity. Here, we applied a serotype-specific DENV envelope domain III (EDIII) IgG enzyme-linked immunosorbent assay (ELISA) capable of distinguishing serotype-specific from cross-reactive antibodies to characterize humoral responses in primary and secondary DENV infections from Laos (Southeast Asia) and Colombia (South America), respectively. While serotype-specific IgG responses targeting the acutely infecting serotype could be readily detected for most primary infections in samples taken approximately three weeks post symptom onset, serotype-specific IgG responses in secondary infections were strongly dominated by the previously encountered serotypes, with comparatively weaker responses directed against the current infecting virus. This pattern is indicative of original antigenic sin, a phenomenon in which the immune system preferentially recalls and amplifies memory responses generated during a first infection in response to a subsequent infection with a closely related pathogen. These findings demonstrate that original antigenic sin is an intrinsic feature of DENV humoral immunity and can be leveraged to infer prior infecting serotypes in secondary infections. Serotype-specific EDIII IgG profiling thus provides a powerful tool to trace past DENV exposures across diverse cohorts and epidemiological settings, with important implications for surveillance, vaccine evaluation, and understanding immune dynamics in endemic regions.

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EVALUATING MYXOVIRUS RESISTANCE PROTEIN A-BASED RAPID TESTING COMBINED WITH PATHOGEN SEQUENCING FOR ARBOVIRAL AND INCIDENTAL VIRAL INFECTION SURVEILLANCE IN SENEGAL

Mouhamed Kane[1], Safietou Sankhe[1], Bocar Sow[2], Marie Henriette Dior Pasteur NDIONE[3], Oumar Faye[1], Cheikh Loucoubar[1], Gamou Fall[4], Cheikh Tidiane Diagne[1], Moussa Moise Diagne[1]

[1]Institut Pasteur de Dakar, Dakar, Senegal, [2]Dakar, Senegal, [3]Dakar, DK, Senegal, [4]Institut Pasteur Dakar, Dakar, Senegal

Accurate differentiation between viral and bacterial infections remains difficult in resource-limited, arbovirus-endemic settings, contributing to diagnostic uncertainty and inappropriate antibiotic use. Myxovirus resistance protein A (MxA), an interferon-induced host biomarker, may provide a pathogen-agnostic approach to improve rapid diagnosis and clinical triage. This study evaluated the performance of an MxA rapid diagnostic test (RDT) using archived samples from febrile patients collected during dengue and chikungunya outbreaks in Senegal. A total of 171 blood samples from patients with acute febrile illness were tested using an MxA RDT and RT-qPCR assays targeting dengue virus (DENV) and chikungunya virus (CHIKV). Samples with discordant results (MxA-positive, RT-qPCR-negative) were further analyzed using metagenomic and hybrid-capture Illumina sequencing to identify potentially missed viral infections. Viral sequences were characterized using maximum-likelihood phylogenetic analysis to determine lineage placement. The MxA RDT showed moderate-to-high sensitivity (70.0-85.1%, depending on virus) and moderate specificity (70.2%) for detecting primary arboviral infections. Sequencing of discordant samples revealed previously undetected pathogens, including DENV serotype 3 (genotype III), parvovirus B19 (genotype 1), and Torque teno virus (TTV). These findings highlight the ability of host-response biomarkers to identify diverse viral infections beyond the pathogens initially targeted by PCR assays. Interpretation Because MxA persists longer than viral RNA, it can help detect recent infections missed by PCR. When combined with sequencing, this approach broadens the diagnostic window, improves patient triage, and supports the identification of underdiagnosed viral pathogens in high-diversity settings. In conclusion, integrating simple host-biomarker diagnostics such as the MxA RDT with pathogen sequencing could strengthen surveillance, improve outbreak preparedness, and expand access to timely viral diagnosis in resource-limited regions.

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COMPLEMENT-MEDIATED VIROLYSIS DEFINES CROSS-REACTIVE ANTIBODY FUNCTION FOLLOWING YELLOW FEVER VIRUS IMMUNITY

Prince Baffour Tonto, Bobby Brooke Herrera

Department of Medicine, Division of Allergy, Immunology, and Infectious Diseases and Child Health Institute of New Jersey, Rutgers Robert Wood Johnson Medical School; Rutgers Global Health Institute, Rutgers University, New Brunswick, NJ, United States

Pre-existing immunity to orthoflaviviruses shapes the outcome of subsequent infections via cross-reactive immune responses that can mediate either protection or pathogenesis. While yellow fever virus (YFV) vaccination or exposure elicits broadly cross-reactive antibodies against related orthoflaviviruses, these antibodies are often weakly neutralizing, suggesting that Fc-dependent effector functions may play a central role in modulating disease outcomes. Hence, we evaluated antibody effector functions following YFV-17D immunization in *IFNAR1*$^{-/-}$ mice. Serum collected 30 days post-immunization demonstrated robust antibody-dependent cellular phagocytosis (ADCP), antibody-dependent complement deposition (ADCD), and complement-mediated virolysis (CMVL) against YFV. In contrast, cross-reactive responses against dengue virus serotype 2 (DENV-2) were more limited, with a subset of samples exhibiting ADCP or ADCD activity. Notably, CMVL against DENV-2 was consistently observed across all samples despite no neutralizing activity, identifying complement activation as a dominant cross-reactive effector mechanism. To translate these findings to human disease, we are conducting ongoing studies in a retrospective cohort of pregnant women in Nigeria, where participants are stratified by pre-existing YFV immunity, subsequent arboviral infection (DENV or Zika virus), and pregnancy outcomes defined by the presence or absence of adverse birth outcomes. Prior analyses indicate that pre-existing YFV immunity is linked to a reduced likelihood of adverse infant outcomes, although the immunologic mechanisms remain unknown. Hence, we are profiling Fc-mediated antibody functions, including ADCP, ADCD, and CMVL, in maternal samples to determine how the quality of cross-reactive antibody responses associates with clinical outcomes after secondary infection. Together, these studies identify CMVL as a key feature of YFV-induced cross-reactive antibody responses and establish a translational framework to assess how antibody effector function may shape disease severity and pregnancy outcomes in endemic populations.

6200

DECADE-LONG ANTIGEN-RESOLVED AND FUNCTIONAL ANTIBODY TRAJECTORIES AMONG EBOLA VIRUS SURVIVORS, HOUSEHOLD CONTACTS, AND COMMUNITY CONTROLS IN EASTERN SIERRA LEONE

Mambu Momoh

Tulane University School of Medicine, New Orleans, LA, United States

Ebola virus disease (EVD) survivors provide a rare window into the durability and thepattern of natural immunity to a high-consequence pathogen. Most studies have limitedfollow-up and do not resolve whether Ebola virus (EBOV)-specific antibody responsesdecline monotonically or show heterogeneous waxing and waning over time. We areconducting a descriptive longitudinal serologic study of EBOV immunity in survivors ofthe 2013-2016 epidemic in Eastern Sierra Leone, with household contacts and pre-Ebola community controls as comparators. The study includes antigen-resolved bindingresponses to EBOV glycoprotein (GP) and nucleoprotein (NP) and functionalneutralizing antibody analyses. Using an anti-EBOV GP IgG ELISA with a predefinedpositivity cutoff and reporting titers in relative units per milliliter, we analyzed 827samples from 155 survivors collected across multiple timepoints from 2014-2024.Preliminary anti-GP results showed 784/827 (95.0%) survivor samples wereseropositive, 38/827 (4.6%) were negative, and 5/827 were indeterminate. Among 43survivors with paired 2016 and 2024 samples, 42/43 (98%) remained anti-GPseropositive, and more than 70% showed increased IgG titers over time rather thanuniform decline. By contrast, only 79/1,331 (6.0%) samples from 383 householdcontacts were anti-GP seropositive across timepoints, and all 100 pre-Ebola communitycontrol samples were anti-GP negative. These findings support durable anti-GPpersistence up to a decade after infection and suggest heterogeneous, non-monotonichumoral trajectories in a substantial subset of survivors. Ongoing anti-NP andneutralization analyses will determine whether these patterns are antigen-specific andfunctionally relevant, with implications for survivor immunity, vaccine benchmarking, andserologic interpretation.

6201

NOVEL ZIKA VIRUS VACCINE DESIGN BASED ON ENVELOPE DOMAINS I & III

Letzibeth Mendez-Rivera[1], Vincent Dussupt[1], Jaime L. Jensen[1], Angélica Peña Rosado[2], Marissa Donofrio[2], Jill Pflugheber[2], Rajeshwer S. Sankhala[1], Wei-Hung Chen[1], Bonnie M. Slike[1], Annika Schmid[1], Ursula Tran[1], Lily Metzger[1], Caroline E. Peterson[1], Amelia K. Pinto[2], Sandhya Vasan[3], Natalie D. Collins[4], Aaron Farmer[4], Nelson L. Michael[4], M. Gordon Joyce[1], James D. Brien[2], Shelly J. Krebs[4]

[1]Walter Reed Army Institute of Research, Henry M. Jackson Foundation for the Advancement of Military Medicine, Silver Spring, MD, United States, [2]University of Kentucky, Lexington, KY, United States, [3]Henry M. Jackson

Foundation for the Advancement of Military Medicine, Bethesda, MD, United States, [4]Walter Reed Army Institute of Research, Silver Spring, MD, United States

There are currently no licensed preventive countermeasures against Zika virus (ZIKV). Vaccine candidates developed through phase 1 trials have been based on the full-length envelope glycoprotein (E), which presents both desirable and undesirable antigenic determinants. Among the latter, the conserved fusion loop epitope within E domain II (DII) is a major target for flavivirus cross-reactive and poorly neutralizing responses that may promote antibody-dependent enhancement (ADE). We developed a rationally designed immunogen composed of domains I and III (DI-DIII) of ZIKV E, excluding DII, while retaining epitopes recognized by potent neutralizing antibodies, such as the DIII lateral ridge and the DI/DIII interface. Structural analysis revealed that the recombinant DI-DIII protein conserves its native conformation and mode of interaction with MZ4, a potent ZIKV protective antibody recognizing the DI/DIII interface. To test whether immunization with DI-DIII could protect *in vivo*, Ifnar1-/- mice were vaccinated twice at day 1 and 21 with 5 µg of either ZIKV DI-DIII, full-length E and DIII and challenged at day 49. Only mice immunized with DI-DIII and E were protected, with > 85% of the animals surviving the lethal challenge. Serological analyses indicated that all immunized groups mounted ZIKV binding responses of similar magnitude, but ZIKV neutralization titers were significantly higher in the DI-DIII and E groups, correlating with protection. In DI-DIII immunized animals, serum depletion with DIII reduced binding and ZIKV neutralization titers by two-fold, indicating that functional responses against DIII and the DI/DIII interface were elicited. Weak but detectable cross-reactivity to other flaviviruses was observed from all groups. To assess the risk of ADE, mouse sera were tested for enhancement of dengue virus (DENV) infection *in vitro*. Sera from DI-DIII vaccinated mice demonstrated a much-reduced capacity to enhance DENV1-4 infection compared to sera from mice vaccinated with E. This study identifies DI-DIII as a promising immunogen able to focus antibody responses on key ZIKV protective epitopes while minimizing ADE risk.

6202

LONGITUDINAL ANTIBODY RESPONSES TO DENGUE SEROTYPES IN RESIDENTS IN PIURA, PERU

Peifang Sun[1], Carolina L. Guevara[2], Ying Cheng[3], Li Pan[4], Corey A. Balinsky[5], Isabella Fox[5], Sarah A. Jenkins[6], Claudia M. Guezala[2]

[1]Naval Medical Research Command, Silver Spring, MD, United States, [2]U.S. Naval Medical Research Unit SOUTH, Lima, Peru, [3]Leidos, Inc., Reston, VA, United States, [4]Henry Jackson Foundation, Bethesda, MD, United States, [5]Henry Jackson Foundation, Silver Spring, MD, United States, [6]Uniformed Services University, Bethesda, MD, United States

The northern coastal region of Piura is a historical epicenter for dengue virus (DENV) and the disease has affected hundreds of thousands of residents. A substantial proportion of DENV infections are asymptomatic, yet these individuals can contribute to the transmission cycle. To better characterize the population-level immunity, we conducted a prospective longitudinal seroprevalence study in Piura from 2019 to 2022. A cohort of 1,885 residents was enrolled and followed for 4 years. Febrile cases were confirmed by PCR. Annual seroprevalence was 57.28% in 2019, 45.94% in 2021, and 49.48% in 2022. Antibodies (Ab) to NS1, neutralization (Neut), and Ab-dependent enhancement (ADE), were assessed to assess immune status and function. Serological profiling distinguished four subpopulations: 1) Unexposed, (UN); 2) Seropositive at enrollment with no significant changes of Ab titters over time (Previously Exposed, PrE); 3) Asymptomatically infected who seroconverted during the study (CONV); 4) PCR-confirmed actively infected febrile cases (INF). Both CONV and INF demonstrated sharp increases in IgG and Neut. In contrast, the PrE group displayed the lowest Neut but the highest ADE. E-IgG levels predicted Neut positivity with 80% accuracy but did not predict Neut titters. Both E-IgG and Neut titters exhibited a bell-curved correlation with ADE. Immune responses following both symptomatic and asymptomatic DENV infections significantly boosted Neut and lowered ADE. However, a large, previously exposed population in Piura maintained a high-risk immune profile of low Neut and high ADE, indicating vulnerability to severe disease. Further research is warranted to investigate the mechanisms sustaining this profile, including possible Ab cross-reactivity among flaviviruses, such as Yellow Fever and Zika viruses, via vaccination or co-circulation.

6203

INVESTIGATING THE SEROTYPE-SPECIFIC INNATE IMMUNE RESPONSES TO DENV INFECTION IN HUMAN SKIN EXPLANTS

Maris S. Wilkins[1], Priscila MS Castanha[1], Ernesto T.A. Marques[2]

[1]University of Pittsburgh, Pittsburgh, PA, United States, [2]Univ of Pittsburgh, Pittsburgh, PA, United States

Dengue virus (DENV), the most prevalent arbovirus worldwide, is a flavivirus with four known circulating serotypes (DENV 1-4), all of which can cause a wide spectrum of disease. Disease severity is influenced by a wide range of factors, including history of a prior infection and the infecting serotype. DENV3 is unique amongst the serotypes for its ability to cause severe disease upon primary infection, with epidemiological studies showing that primary and secondary DENV3 infections have similar rates of disease severity. Early tissue level events that underlie DENV3 disease and its propensity for causing severe disease are currently unknown. In this study, we used a well characterized skin explant model to explore these early host-virus interactions at the primary site of DENV infection. Abdominal skin from individuals undergoing elective cosmetic procedures was obtained from hospitals in the Pittsburgh area and infected with DENV1 16007, DENV2 16681, DENV3 16662, and DENV4 1036. Tissue was collected at 1, 3, and 5 days post infection and cryopreserved in 30% sucrose before sectioning and being stained with anti-NS3, CD163, CD207, and CD1c antibodies. Images were acquired at 40x on a confocal microscope and analyzed in NIS Elements for the total area of tissue that was positive for the marker of interest. All 4 viruses productively infected cells of both the epidermis and dermis, with DENV3 displaying the highest level of infection and significantly less recruitment of immune cells such as macrophages and Langerhans cells to the site of infection compared to the other 3 viruses. However, tissue infected with DENV3 displayed the highest proportion of infected immune cells, with close to 80% of immune cells detected being positive for NS3. To further investigate these differences, we will continue to look at infection of skin cells such as keratinocytes and fibroblasts that are known to play a role in innate immune signaling and quantify the viral genomic copies by PCR. Additionally, we will use flow cytometry to characterize the cell types and infection status of cells that have migrated out of the tissue over the course of infection.

6204

MYELOID CELL DYNAMICS FOLLOWING DENGUE 4 LIVE VIRUS HUMAN CHALLENGE

Cristiana Cairo[1], Sigrid Camacho Ortega[2], David Thomas Rach[3], Joel V. Chua[2], Kirsten E. Lyke[4]

[1]Department of Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, MD, United States, [2]Institute of Human Virology, University of Maryland School of Medicine, Baltimore, MD, United States, [3]University of Maryland, Baltimore, Baltimore, MD, United States, [4]Center for Vaccine Development and Global Health, University of Maryland School of Medicine, Baltimore, MD, United States

Dengue human infection models (DHIM) are powerful tools to down-select dengue vaccine candidates, study pathogenesis, and assess immune responses to the virus with small cohorts of volunteers. We optimized 27-28 color spectral flow cytometry panels for a comprehensive analysis of myeloid (all major monocytes/DC subsets) and B cell dynamics interrogating longitudinal specimens from 14 participants challenged with underattenuated Dengue virus serotype 4 (DENV-4) strain H241. Six of 14 original participants underwent a homologous rechallenge (one year after the primary challenge), with biospecimens collected at identical timepoints. For each participant, PBMC obtained before challenge, one day after the onset of viremia, one day after viremia cessation, 90, and 180 days after challenge were processed concomitantly, along with healthy control PBMC included for normalization across experiments. Using a semi-supervised

analytical approach, we observed perturbation in both B and myeloid cell compartments after primary challenge, with no immunologic responses after rechallenge. Elevated plasmablast proportions were noted at viremia cessation in all participants except one (who displayed a delayed onset of viremia and the mildest symptoms), accompanied by increased expression of CD71. Monocytes frequencies were lower after cessation of viremia versus before challenge and normalized by D90, with differences driven by the intermediate and non-classical subsets. A similar trend was observed for the frequency of CD14+DC3, known to have pro-inflammatory features, and for the CD56+ component of DC2 and DC3. Several markers were significantly upregulated upon resolution of viremia within monocyte subsets, including CD89, CD38, CD163, and CD64/FcγRI. Of note, CD64 was significantly upregulated within DC2, DC3 and CD14+DC3 upon cessation of viremia. Overall, our in-depth immunologic study, including early time points after dengue infection, validated the DHIM-4 as consistent with immunological findings seen in natural dengue infection and provided new information about myeloid subsets that had not been previously investigated.

6205

MULTIPLEX-DERIVED HUMORAL IMMUNE SIGNATURES PREDICT NEUTRALIZATION AND DISTINGUISH MPXV INFECTION FROM VACCINATION

Cynthia G. Lorang[1], Neeraja Venkateswaran[2], Kodumudi Venkateswaran[3], Christopher N. Mores[4]

[1]The Milken Institute School of Public Health, The George Washington University, Washington, DC, United States, [2]Tetracore Inc, Rockville, MD, United States, [3]Tetracore Inc., Rockville, MD, United States, [4]George Washington University, Washington, DC, United States

Monkeypox virus (MPXV), an *Orthopoxvirus* (OPXV) related to variola and vaccinia viruses, re-emerged globally in 2022, exposing gaps in scalable rapid immunity assessment and real-time outbreak response capacity. *OPXV* cross-reactivity complicates serologic interpretation in mixed-immunity populations. Neutralizing antibodies (nAb) against MPXV wane within 6-12 months, making scalable alternatives to neutralization assays essential. We hypothesized that MPXV infection and vaccination induce distinct humoral immune signatures defined by antigen-specific binding and nAb kinetics that classify exposure source and predict functional neutralization. To test this, we used a novel xMAP-based, 8-plex anti-*OPXV* serology assay to characterize IgG profiles from the 2022-23 outbreak across 24 acute MPXV cases (2-12 days), 8 longitudinal samples (up to 15 months), 3 vaccinated, and 2 unvaccinated controls, alongside $FRNT_{50}$ titers. nAbs ($FRNT_{50}$ ≥32) were detected in only 3/24 acute samples, indicating limited early functional immunity despite robust binding to MPXV antigens B6R and A35 (MFI >6000; >1000), demonstrating that multiplex detection precedes functional neutralization. Longitudinal nAb titers followed classic kinetics, peaking at 6 months ($FRNT_{50}$ = 75) and declining by 15 months, with a shift toward IMV-dominant responses including M1R and B6R. MPXV-infected samples showed strong binding to B6R and A35 (>6000; >1000 MFI), distinct from unvaccinated controls with minimal binding and no neutralization. Vaccinated controls differed by platform, with JYNNEOS vaccination showing binding without nAbs and variola vaccination showing high cross-reactive binding to A33R and B6R (MFI > 10500; > 3500) with variable nAb responses. ROC analysis using $FRNT_{50}$ ≥1:32 identified thresholds predictive of nAb status. A33R, M1R, B6R, and A35 achieved perfect classification (AUC=1.00, p<0.0001). Multiplex-derived humoral immune signatures distinguish MPXV infection from vaccination and predict nAb status, providing a scalable framework for outbreak diagnostics and real-time immune surveillance in heterogeneous populations.

6206

CHIKUNGUNYA VACCINE ACCEPTABILITY AMONG RESIDENTS OF PONCE, PUERTO RICO, 2024-2025

Sarah Anne Anne J. Guagliardo[1], Chelsea Major[2], Dania Rodriguez[3], Vanessa Rivera-Amill[4], J. Erin Staples[5], Laura Adams[6], Susan Hills[1]

[1]Centers for Disease Control and Prevention, Fort Collins, CO, United States, [2]Centers for Disease Control and Prevention, San Juan, PR, United States, [3]San Juan, PR, United States, [4]Ponce Medical School Foundation, Inc., Ponce, PR, United States, [5]Centers for Dis Control & Prevention, Fort Collins, CO, United States, [6]CDC, San Juan, PR, United States

Recent availability of chikungunya vaccines has expanded prevention options for this mosquito-borne disease, which can cause acute febrile illness, prolonged arthralgia, and may lead to severe complications including death. Puerto Rico experienced a large chikungunya outbreak in 2014 and remains at risk for future transmission. During 2024-2025, we surveyed participants aged ≥18 years of the ongoing Communities Organized to Prevent Arboviruses (COPA) community-based cohort study in Ponce, Puerto Rico to assess the acceptability of a hypothetical chikungunya vaccine provided free or at low cost (≤10 USD). Among 2,437 participants, 70.7% reported willingness to be vaccinated, 22.6% were unwilling, and 6.7% were unsure. In multivariable logistic regression adjusted for sex and geographic cluster, vaccine acceptance was lower among participants aged 31-50 years (adjusted Odds Ratio [aOR]: 0.51, 95% confidence interval [CI]: 0.38-0.70) and >50 years (aOR: 0.67, 95% CI:0.47-0.96) compared with those aged 18-30 years, and among those with some higher education (aOR:0.50, 95% CI:0.29-0.87) compared with those with less than high school education. The most common reasons for being uninterested or unsure were wanting more information about vaccine safety and side effects (36.3%) and about how the vaccine works (21.6%). These findings highlight high overall acceptability but identify key subgroups with lower potential uptake and underscore the need for targeted communication and education strategies to support effective chikungunya vaccination programs in settings at-risk for transmission.

6207

BREADTH AND PERSISTENCE OF TAK-003-INDUCED NEUTRALIZING ANTIBODIES AND T CELLS ACROSS AGE, REGION AND BASELINE SEROSTATUS

Sanja Mandaric[1], Tarek El Hindi[2], Xavier Saez-Llorens[3], Charissa Borja Tabora[4], Heather Friberg[5], Vianney Tricou[1], Nicholas Roubinis[1], Jeffrey Robert Currier[5], Shibadas Biswal[2]

[1]Takeda Pharmaceuticals International A.G, Zurich, Switzerland, [2]Takeda Vaccines, Inc., Cambridge, MA, United States, [3]Hospital del Niño Dr. José Renán Esquivel, Panama City, Panama, [4]Clinical Research Division, Research Institute for Tropical Medicine, Muntinlupa, Philippines, [5]Walter Reed Army Institute of Research, Silver Spring, MD, United States

TAK-003 is a live-attenuated tetravalent dengue vaccine licensed in multiple countries without a requirement for pre-vaccination serostatus testing. Here we report the vaccine-elicited immune responses after dose 1 and 2 and summarize the long-term persistence in participants throughout the clinical development program, across age, region and baseline dengue serostatus. Immunogenicity was assessed after TAK-003 dose 1 and 2 in multiple Phase 2/3 studies, by measuring neutralizing antibodies (NAbs) to each DENV serotype using a dengue microneutralization test (MNT50) in children, adolescents and adults from endemic regions, and in adolescents and adults from non-endemic regions. DENV-specific T cell responses against non-structural and structural proteins were evaluated after TAK-003 dose 1 and 2 using an interferon-gamma enzyme-linked immunospot (IFN-γ ELISPOT) assay. TAK-003 elicited early tetravalent T cell and NAb responses within 15-30 days after dose 1, irrespective of age group and baseline serostatus, in both dengue endemic and non-endemic regions. At Day 120 (one month after dose 2), 90.9%-100% of dengue baseline seronegative participants across age and region achieved NAb seropositivity against all four serotypes and IFN-γ ELISPOT T cell responder rates against any DENV peptide pool of 98.6%-100%. NAb and T cell

responses were durable and persisted during an observed period of up to 69 and 39 months post dose 1, respectively, regardless of baseline serostatus. In conclusion,TAK-003 induced rapid tetravalent NAb and T cell responses which persisted through observed long-term follow-up across ages and baseline serostatus in endemic and non-endemic populations. The breadth and durability of TAK-003-elicited immunity across these populations are consistent with the vaccine's observed clinical protection and safety profile in pivotal efficacy and real-world studies.

6208

FROM INNOVATIVE METHODS TO EFFECTIVE IMPLEMENTATION STRATEGIES: THE NESTED CASE-CONTROL EFFECTIVENESS STUDY OF TAK-003 AGAINST DENGUE HOSPITALIZATION (DEN-401)

Randee J. Kastner[1], Htar Htar Nwe[2], Kai Fern Chan[2], Debbie Mendoza[2], Chakrarat Pittayawonganon[3], Sri Rezeki Hadinegoro[4], Rafdzah Ahmad Zaki[5], Prabda Praphasiri[3], Nina Dwi Putri[6], Manja Brose[7], Michael Hellwig[1], Marialodovica Chiesa[1], Tim Rindfleisch[8], Kelley J. Moss[8], Vijaya Golamaru[7], Laurence Baril[1], John Weil[1]

[1]Takeda Pharmaceuticals International AG, Zurich, Switzerland, [2]Takeda Pharmaceuticals International AG, Singapore, Singapore, [3]Ministry of Public Health, Nonthaburi, Thailand, [4]Department of Child Health, Faculty of Medicine, University of Indonesia, Cipto Mangunkusumo hospital, Jakarta, Indonesia, [5]Center for Epidemiology and Evidence-Based Practice, Department of Social and Preventative Medicine, Faculty of Medicine, Universiti Malaya, Kuala Lumpur, Malaysia, [6]Cipto Mangunkusumo Hospital, University of Indonesia, Jakarta, Indonesia, [7]Takeda Development Center Americas, Inc., Cambridge, MA, United States, [8]Takeda Vaccines Inc., Cambridge, MA, United States

Takeda's tetravalent dengue vaccine, TAK-003, has demonstrated safety and efficacy in the DEN-301 trial (NCT02747927). The vaccine is recommended by the WHO Strategic Advisory Group of Experts in areas with high dengue transmission and is approved in >40 countries. However, uncertainty remains regarding the efficacy of dengue virus (DENV)-3 and DENV-4 in hospitalized baseline seronegative individuals. The nested case-control phase 4 study, DEN-401 (NCT06843226), began in 2025 in collaboration with health authorities and academic institutions across dengue-endemic countries in Southeast Asia. It was designed to assess TAK-003's effectiveness against hospitalized virologically confirmed dengue (VCD) by serotype and by baseline serostatus. Here, we provide the study's implementation strategies and overall progress. In total, 70,000 participants (6-12 years old) are being enrolled. At enrollment, blood samples are being collected from all participants to determine baseline dengue seropositivity among cases and matched controls. Hospital-based active surveillance is being conducted to identify hospitalized VCD, including severe cases. Each identified VCD case is randomly matched to 10 controls based on age and geographic area. Enrollment has begun in Thailand (March 5, 2025; n=35,000) and Indonesia (September 22, 2025; n=30,000); Malaysia (n=5000) is expected to start enrollment in April 2026. As of [March 16, 2026, a total of 59,022 participants have been enrolled, which is >84% of the cohort. Enrollment was completed in Thailand as of December 26, 2025, while enrollment in Indonesia is ongoing (n=24,022 as of March 16, 2026). Data quality will be ensured through operational tools, bioanalytical management, and cross-functional data review. All participants will be followed for 3 years, with final results expected by 2029 after data collection ends. Our robust implementation strategies provide a foundation for high-quality data generation to support decision-making bodies such as national immunization technical advisory groups and immunization directorates within ministries of health.

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SAFETY OF THE DENGUE VACCINE TAK-003 AND FACTORS ASSOCIATED WITH ADVERSE EVENTS IN TRAVELLERS FROM NON-ENDEMIC AREAS: A REAL-WORLD PROSPECTIVE MULTICENTRE PHARMACOVIGILANCE COHORT STUDY IN SPAIN

Cesc Bertran Cobo[1], Meritxell Saloni[1], Lluis Valerio[2], Judith Villar[3], Victoria Lopez Lopez[4], Elisabet Jurado[5], Maria Luisa Machado[6], Daniela Perez-Leon[7], Sarai Cuevas[8], Jose Muñoz[1], **Daniel Camprubí Ferrer**[1]

[1]ISGlobal, Barcelona, Spain, [2]PROSICS Metropolitana Nord, Institut Català de la Salut, Badalona, Spain, [3]Hospital del Mar, Barcelona, Spain, [4]Centre de Salut Internacional i Malalties Transmisibles Drassanes, Vall d'Hebron University Hospital, Barcelona, Spain, [5]Hospital Universitari Arnau de Vilanova, Lleida, Spain, [6]Hospital Parc Taulí, Sabadell, Spain, [7]Hospital Universitari de Bellvitge, L'Hospitalet de Llobregat, Spain, [8]Consorci Sanitari de l'Alt Penedès i Garraf, Vilafranca del Penedès, Spain

TAK-003 is the first dengue vaccine licensed for endemic and non-endemic regions, but real-world safety data in non-endemic travel settings are scarce, particularly for older adults, people with comorbidities, and concomitant vaccinations. We conducted a prospective multicentre pharmacovigilance study in eight Spanish travel clinics, enrolling travellers aged ≥4 years who received TAK-003 (January-December 2024). Participants were followed for adverse events (AEs) after each dose. Logistic regression models assessed associations of age, sex, comorbidities, prior flaviviral infection or vaccination history, and concomitant vaccination with risks of any, systemic, or local AEs. Among 1,028 participants (1,851 TAK-003 doses), 998 (97·1%) completed ≥1 follow-up assessments; 587/998 (58·8%, 95% CI 55·7-61·8) reported AEs. After the first dose, 47·1% (44·0-50·2) reported AEs, most often injection site pain and swelling, headache, or malaise, declining to 34·6% (31·1-38.3) after the second dose. No serious AEs, anaphylaxis, or deaths occurred. Female sex was associated with any AEs (adjusted OR 1·83, 95%CI 1·42-2·37) and local AEs (1·89, 1·44-2·48). Prior dengue was associated with any (1·51, 1·07-2·14) and systemic AEs (1·73, 1·22-2·43). Concomitant flaviviral vaccination was associated with higher odds of systemic AEs (1·64, 1·18-2·28). Age ≥60 years and comorbidities were not associated with increased AE risk. TAK-003 was well tolerated in travellers from non-endemic regions, with no serious AEs reported. Female sex, prior DENV infection, and flaviviral vaccine co-administration were associated with increased reactogenicity; older age, comorbidities, and non-flaviviral vaccine co-administration were not. These findings provide real-world evidence on the tolerability of TAK-003 in travellers from non-endemic regions. Larger studies with extended follow-up are needed to better characterise uncommon and longer-term AEs. This study did not receive any dedicated funding.

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COST-EFFECTIVENESS OF TAK-003 DENGUE VACCINATION IN PUERTO RICO

Maile B. Thayer[1], Zachary J. Madewell[1], Guido Felipe Camargo España[2], Alfonso C. Hernandez-Romieu[3], Joshua M. Wong[1], Michael A. Johansson[4], Laura E. Adams[1], Gabriela Paz-Bailey[1], T Alex Perkins[5]

[1]Centers for Disease Control and Prevention, San Juan, Puerto Rico, [2]University of Notre Dame, Mishawaka, IN, United States, [3]Centers for Disease Control and Prevention, Atlanta, GA, United States, [4]Northeastern University, Boston, MA, United States, [5]University of Notre Dame, Notre Dame, IN, United States

Dengue virus imposes substantial health and economic burdens, but control remains challenging. TAK-003, a tetravalent live attenuated vaccine, has shown sustained protection against symptomatic and hospitalized dengue and is currently recommended in high-transmission settings without pre-vaccination screening. However, its cost-effectiveness in Puerto Rico has not yet been evaluated. We developed a dynamic dengue transmission model calibrated to Puerto Rico data and linked to an economic evaluation.

The model incorporated serostatus- and serotype-specific vaccine efficacy estimates from Phase 3 trial data and simulated four strategies: no vaccination, or vaccination of children (6-16 years), adults (17-60 years), or both (6-60 years) assuming 40% vaccination coverage. Outcomes included hospitalizations, ambulatory cases, deaths, disability-adjusted life years (DALYs), and costs (2025 USD) from healthcare system (direct medical costs; relevant for policymakers, insurers, and hospital administrators) and societal (also includes non-medical and indirect costs; relevant for patients, employers, and the wider economy) perspectives over 10 years. Probabilistic sensitivity analyses assessed cost-effectiveness across willingness-to-pay (WTP) thresholds. Without vaccination, Puerto Rico was estimated to have 201,700 ambulatory cases, 36,700 hospitalizations, 190 deaths and 5,500 DALYs, costing $572 million (healthcare perspective) to $2.6 billion (societal perspective). Vaccinating all ages reduced ambulatory cases by 34%, and hospitalizations, deaths and DALYs by 43-46%. Pediatric vaccination averted fewer cases but was cost-saving ($36 million) from the healthcare system perspective. Adult-inclusive strategies increased costs but were cost-effective at WTP thresholds >$65,000 per DALY averted. From a societal perspective, all vaccination strategies were cost-saving, with savings of $375-884 million. TAK-003 vaccination in Puerto Rico could substantially reduce dengue morbidity, mortality, and economic burden. These findings support consideration of TAK-003 implementation in Puerto Rico.

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N-GLYCAN DELETIONS IN THE LASSA VIRUS GLYCOPROTEIN PRESERVE FUNCTION AND SUPPORTS VACCINE DEVELOPMENT

Christine A. Click, Takeshi Saito, Gavriella Siman-tov, Ruchi Paroha, Slobodan Paessler, Junki Maruyama

University of Texas Medical Branch, Galveston, TX, United States

Lassa fever, caused by Lassa virus (LASV), is a hemorrhagic disease associated with high mortality. There are no approved vaccines, and therapeutics are limited. As such, it poses a significant health threat and economic burden in West African endemic areas, although sporadic cases are reported internationally. The primary target of neutralizing antibodies is the LASV glycoprotein complex (GPC), the virus's sole envelope protein. However, the GPC is densely covered by eleven evenly spaced N-glycans that promote immune evasion via molecular mimicry and shielding key epitopes, likely contributing to the insufficient neutralizing antibody response observed during human infection. In this study, we investigate how these highly conserved N-glycans influence GPC processing, function, and immunogenicity to inform vaccine design. We constructed GPC expression plasmids that prevent glycosylation at each site individually or in sets, then assessed their contributions to protein expression and proteolytic cleavage. Results determined that defined amino acid substitutions in specific locations lead to aberrant cleavage events, although most mutations do not alter protease activity. Regardless, in all instances, GPC translocation to the cell surface was observed by immunofluorescence assays. Confocal microscopy further characterized changes in localization and processing. We evaluated the function of our mutant GPC (mGPC) lacking N-glycans using a single-cycle, pseudotyped vesicular stomatitis virus (VSV). We discovered that all glycans can be removed while retaining normal functional activities, disputing current literature. In fact, at least 45% of glycans can be simultaneously lost without identifiable negative effects on GPC function. Recombinant VSV expressing mGPC or wild-type GPC was used to evaluate antibody responses in immunized mice. Together, our findings challenge current models of LASV N-glycan requirements for processing and reveal system-level tolerance to concerted glycan loss while preserving function. These data support a glycan-focused VSV-based LASV vaccine platform with broad coverage potential.

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PRIOR INACTIVATED JAPANESE ENCEPHALITIS VIRUS VACCINATION INFLUENCES ANTIBODY RESPONSES TO LIVE-ATTENUATED DENGUE VACCINATION

Patrick I. Mpingabo[1], Esther I. Adekomi[1], Lisa A. Ware[1], Monir Hossain[1], Joseph Q. Lu[1], Heather L. Friberg[2], Gregory D. Gromowski[2], Kathryn B. Anderson[1], Stephen J. Thomas[1], Adam T. Waickman[1]

[1]SUNY Upstate Medical University, Syracuse, NY, United States, [2]Walter Reed Army Institute of Research, Silver Spring, MD, United States

Previous exposure to infections or vaccines, known as immune history, can impact how the body responds to subsequent vaccinations. However, the effects of sequential flavivirus vaccinations on antibody responses in humans remain poorly understood. This issue is particularly relevant in areas where both Japanese encephalitis virus (JEV) and dengue virus (DENV) vaccines are administered. In our study, we employed a serological systems approach that integrated competitive epitope mapping (blockade-of-binding assay) and antibody function tests (including virus neutralization and enhancement) to examine how prior vaccination with the inactivated JEV vaccine IXIARO affects antibody responses following receipt of the live-attenuated dengue vaccine CYD-TDV. We observed that people previously vaccinated against JEV produced dengue antibodies that primarily target a highly conserved region of the virus, the fusion loop. These antibodies reacted broadly across different dengue virus serotypes and recognized the Zika virus, but had limited ability to neutralize infection. Additionally, these antibodies were linked with increased activity in lab tests measuring antibody-dependent enhancement, in which antibodies can help the virus infect certain immune cells. By using a modified dengue envelope protein that disrupts the fusion loop epitope, we confirmed that antibodies directed against this region accounted for a large part of the response after sequential vaccination. Importantly, giving the live-attenuated dengue vaccine multiple times reduced this fusion-loop targeting and encouraged antibody responses to other parts of the virus. These results imply that previous vaccination history impacts the quality and focus of antibody responses to dengue vaccines. Gaining a better understanding of how vaccination history and regimens affect these responses may help improve vaccination strategies for dengue and other flaviviruses in endemic regions.

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DEVELOPING AN IMMUNOGENIC AND CROSS-PROTECTIVE FLAVIVIRUS MRNA VACCINE

Megan Nicole Carey, Maxwell A. Ross, Allyson N. Hidalgo, Daniel P. Rehm, Athos Silva de Oliveira, Jeroen Pollet, Jennifer Lynn Spencer Clinton

Baylor College of Medicine, Houston, TX, United States

Flaviviruses cause >400 million infections annually and can cause significant birth defects and severe illness. Climate change is increasing flavivirus outbreak potential, and while vaccines against individual flaviviruses are in development, most are not approved, and none offer cross-protection against viruses with overlapping endemicity. Prior studies indicate that ZIKV envelope (E) protein may protect against related flaviviruses. This study aims to develop a cross-protective flavivirus mRNA vaccine targeting the highly conserved flavivirus E protein and assess immunogenicity in mice. Three ZIKV E mRNA candidate vaccines were generated with secreted, membrane-bound, or ubiquitin signal sequences to direct differential localization or processing. In vitro expression was confirmed by immunofluorescence and Western blotting. Vaccines were formulated in lipid nanoparticles as monovalent or multivalent constructs. C57BL/6 mice (n=12/group) received prime and boost immunizations of E constructs or eGFP controls 21 days apart. Sera were collected prior to each immunization. Sera and spleens were harvested 21 days post-boost. Splenocytes were stimulated with E peptide pools and T-cells were quantified by flow cytometry. Total ZIKV E-specific IgG antibodies were quantified by ELISA. Splenocytes from the secreted and membrane-bound E vaccine groups generated significantly higher antigen-specific

Th1 CD8+ T-cell responses and elicited 3-fold higher total E-specific IgG titers compared to controls ($p<0.05$). We are currently performing a ZIKV challenge study in IFNAR$^{-/-}$ mice and characterizing neutralizing antibody titers against ZIKV and other flaviviruses by plaque reduction neutralization tests as a measure of cross-protective potential. Results indicate that our ZIKV E protein mRNA vaccine elicits robust cell-mediated and humoral immunity in mice. Future studies will assess protection against ZIKV, dengue, and West Nile virus infections and identify correlates of protection. The goal of this work to generate an effective, safe, cross-protective flavivirus vaccine that can be readily adaptable for outbreaks.

6214

ENHANCING, CONTROLLING, AND STERILIZING DENGUE IMMUNITY AND THE DEVELOPMENT OF BROADLY PROTECTIVE RESPONSES

Rosemary A. Aogo[1], Camila D. Odio[1], Saba Firdous[1], Charlie Voirin[1], Silvia Blanco-Rivera[1], Kelsey Lowman[1], Nhyira Asante[1], Ysabelle Broderson[1], Keerthi Konda[1], Chloe Moon Hasund[1], Amparo Martinez-Perez[2], Patrick Ilunga Mpingabo[1], Melissa A. Law[3], Caitlin Jarvis[3], Viviane Callier[4], Sally Hunsberger[5], Maria Abad Fernandez[6], Aravinda de Silva[6], Robbie Kattappuram[7], Gitanjali Bhushan[1], Christina Yek[8], Jessica E. Manning[9], Anna P. Durbin[10], Jeffrey I. Cohen[11], Daniela Weiskopf[2], Stephen Whitehead[12], Leah Katzelnick[1]

[1]Viral Epidemiology and Immunity Unit, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States, [2]Center for Vaccine Innovation, La Jolla Institute for Immunology, La Jolla, CA, United States, [3]Clinical Research Directorate, Frederick National Laboratory for Cancer Research, Frederick, MD, United States, [4]Clinical Monitoring Research Program Directorate, Frederick National Laboratory for Cancer Research, Frederick, MD, United States, [5]Division of Clinical Research, Biostatistics Research Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States, [6]Department of Microbiology and Immunology, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States, [7]Department of Pharmacy, NIH Clinical Center, National Institutes of Health, Bethesda, MD, United States, [8]Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States, [9]National Institute of Allergy and Infectious Diseases, Washington, DC, United States, [10]Johns Hopkins Bloomberg School of Public Health, Takoma Park, MD, United States, [11]Medical Virology Section, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States, [12]Arbovirus Vaccine Research Section, Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States

Dengue is the canonical viral disease for which immune history predicts protective versus pathogenic responses and immunogenicity. Yet, due to limitations in animal models and clinical presentation after peak viremia, how pre-infection and early immune responses affect dengue outcomes is not confirmed. We conducted a phase 1 clinical trial with 45 healthy adults to test whether secondary infection challenge with a heterotypic, full-length, attenuated virus (rDENV3Δ30/31-7164) increases viremia and immunogenicity compared to primary and tertiary infection. Viremia was associated with more, but still mild, clinical signs and symptoms, and secondary infection predicted greater viremia and neutralizing antibodies. However, those with the highest baseline enhancing antibodies experienced delayed inflammatory and adaptive activation, the highest viremia, strong acute immune responses, but waning of potent CD8+ T cells and antibodies. Baseline antibodies to non-structural protein 1 (NS1) of multiple serotypes predicted early interferon and balanced immune activation, viremia control, and development of enduring, potent B and T cells, revealing how vaccines can induce broad long-lasting protection. Finally, these potent antibody and T cell profiles at baseline predicted sterilizing immunity. This work demonstrates that controlled human challenge can delineate coordinated versus dysregulated acute responses and effects on immunogenicity, informing therapeutic and vaccine strategies for dengue and other viral diseases. In ongoing work, we are conducting in-depth immunophenotyping of peripheral blood mononuclear cells from this trial and have developed a mathematical framework that models the interactions among uninfected and dengue virus-infected myeloid cells, NK cells, neutrophils, T and B lymphocytes, antibodies, and cytokines. This framework will elucidate how recall responses shape de novo acute responses, highlighting the effects of innate immunity on adaptive responses and providing insights into the distinct immune profiles observed here.

6215

EVALUATION OF CROSS PROTECTIVE IMMUNITY OF A COMMERCIAL FLAVIVIRUS VETERINARY VACCINE AGAINST JAPANESE ENCEPHALITIS VIRUS IN DOMESTIC SWINE

Audrey M. Matheny[1], Nicole E. Ostrander[1], Susan M. Hettenbach[1], Ashlie M. Ake[1], Annika T. Lindt[1], Stephen Higgs[2], Dana L. Vanlandingham[1]

[1]Kansas State University, Manhattan, KS, United States, [2]Biosecurity Research Institute, Manhattan, KS, United States

Japanese encephalitis virus (JEV) is a mosquito-borne orthoflavivirus of significant public health concern, capable of causing severe disease in humans and reproductive issues in swine. The recent outbreak of JEV in mainland Australia in which commercial swine were particularly affected, highlights the ongoing risk of JEV introduction to new areas including the United States (U.S.) and the need to continue development of effective countermeasures. Whilst sensitive diagnostics are available, there is no JEV specific antiviral treatment nor U.S. Department of Agriculture (USDA) approved JEV vaccine available for veterinary use in the U.S. However, there are several USDA approved vaccines for West Nile virus (WNV), a closely related virus, currently available. We evaluated the cross protective immunity of an approved WNV vaccine against JEV utilizing a domestic swine challenge model. Groups of four-week-old piglets were vaccinated following the manufacturer's recommended dosing regimen: primary dose with a booster 21 days later. Mock vaccinated control groups were included. Piglets were monitored daily for post-vaccination reactions and weighed weekly to monitor growth. Serum was collected on Day 0 prior to vaccination, and at four timepoints prior to challenge to monitor development of immune response. Vaccinated pigs and a subset of mock vaccinated pigs were challenged with JEV using intradermal inoculation on Day 36 post primary vaccination. Another subset of mock vaccinated pigs was mock challenged as negative controls. Serum, nasal, and oral swabs were collected from pigs on Day 35, and daily after challenge starting on day post infection (DPI) 1 - 7, DPI 9, DPI 11, and DPI 14. A predetermined subset of pigs was terminated on DPI 3 with necropsy tissues collected to determine viral spread during the acute phase of infection. The remaining pigs were terminated on DPI 14. Demonstration of successful cross protection against JEV using this commercially available WNV vaccine will provide evidence that this vaccine could be used to protect domestic swine in the event of a JEV introduction to the U.S.

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DEVELOPMENT OF THE DENGUE-SPECIFIC B CELL RESPONSE IN LYMPH NODES AND PERIPHERAL BLOOD FOLLOWING MONOVALENT VACCINATION

Kelsey E. Lowman[1], Rosemary A. Aogo[1], Keerthi N. Konda[1], Saba Firdous[1], Silvia E. Blanco Rivera[1], Chloe M. Hasund[1], Charlie J. Voirin[1], Patrick I. Mpingabo[1], Ysabelle Broderson[1], Nhyira Asante[1], Gitanjali Bhushan[1], Richard Apps[2], Jesse Polanco[2], Melanie Cohen[3], Julie Laux[3], Ashley Gallagher[4], Michael Kassin[4], Elliot B. Levy[4], Cali R. Lubrant[4], Ifechi N. Ukeh[4], Barbara Blacklaws[5], Jonathan L. Heeney[5], Iyadh Douagi[3], Bradford J. Wood[4], Camila D. Odio[1], Leah C. Katzelnick[1]

[1]Viral Epidemiology and Immunology Unit, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States, [2]Center for Human Immunology, National Institutes of Health, Bethesda, MD, United States,

[3]Flow Cytometry Section, Research Technologies Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States, [4]Interventional Radiology, Clinical Center, National Institutes of Health, Bethesda, MD, United States, [5]Department of Veterinary Medicine, University of Cambridge, Cambridge, United Kingdom

Secondary dengue virus (DENV) infection is associated with the development of broadly protective immunity, though it is unclear whether this results from recruitment of recalled, cross-reactive B cells to germinal centers (GC) or from *de novo* B cells responses. We conducted a DENV3 vaccine trial to model sequential infection in 45 individuals across different DENV immune histories (naïve, monotypic, and polytypic). To describe differential B cell responses across DENV histories and to capture GC reactions, we implemented fine needle aspirate sampling of lymph nodes (LN) to compare DENV-specific B cells in peripheral blood and LNs of three naïve and six immune participants across pre- and post-vaccine (days 15, 28, and 57) timepoints. From flow cytometric analyses using DENV envelope dimer probes, we observed recall of DENV-specific B cells as seen with an increased prevalence of DENV-reactive IgG plasmablasts in the blood and a predominately cross-reactive CXCR3+ B cell population in LNs of immune individuals on day 15. Higher DENV-specific CXCR3+ B cell and cross-reactive IgM memory B cell counts in day 15 LNs associated with higher quality serum antibody titers on day 57, suggesting recall of DENV-specific B cells to LNs was important in the improvement of the convalescent antibody repertoire. We found imbalanced dark zone:light zone B cell ratios after vaccination, where light zone cells outnumbered dark zone cells in both immune and naïve individuals. This suggests dark zone re-entry or T follicular helper cell (Tfh) signaling may be restricted, which could result in impaired GC responses and reduced affinity maturation. Despite this, higher day 15 Tfh frequencies associated with increased serum neutralization breadth on days 28 and 57, indicating T cell selection was important in the diversification of neutralization activity. Our ongoing work characterizing circulating and LN cell populations responsible for initiating and driving GC reactions underlying differential vaccine responses by multimodal single-cell sequencing will provide valuable insight to our basic understanding of DENV-specific B cell and antibody responses.

6217

PRECLINICAL DEVELOPMENT OF NOVEL REASSORTANT LASSA VACCINE ML29-SF

Peter Pushko[1], Igor S. Lukashevich[2], Dylan M. Johnson[3], Irina Tretyakova[1]

[1]Medigen, Inc, Frederick, MD, United States, [2]University of Louisville, Louisville, KY, United States, [3]Sandia National Laboratories, Livermore, CA, United States

Lassa fever (LF) virus is a human pathogen causing outbreaks in West Africa and periodic imported cases occurring in the U.S., European, and other countries by infected travelers. There is no approved vaccine, and according to the WHO, development of a preventive vaccine for LF is one of the highest priorities. Replication-competent vaccines provide long-term broad immunity with a single dose, which makes them uniquely suitable to rapidly contain outbreaks and epidemics such as LF. The experimental vaccine ML29 was developed by arenavirus reassortant technology and contains the L genomic RNA segment derived from nonpathogenic Mopeia virus (MOPV), and the S genomic RNA segment from Lassa virus (LASV). In addition to reassortment, multiple mutations distinguish ML29 genome from the parental MOPV and LASV. Vaccine has been adapted to serum-free (SF) manufacturing to manufacture under current Good Manufacturing Practices (cGMP). ML29-SF vaccine was characterized phenotypically (growth kinetics, plaque morphology), biochemically, immunologically, genetically (NGS), and other methods. A 2-week repeat dose toxicity study was performed in guinea pigs under Good Laboratory Practice (GLP) regulations to assess the local and systemic toxicological effects. Following an intramuscular (IM) or subcutaneous (SC) injection of 10^4 PFU of ML29-SF vaccine at the start of the study, with a second dose 15 days later, no toxicological response attributable to the vaccine was observed. Overall, this single-dose vaccine was locally and systemically well tolerated even after a repeat administration, confirming the high level of safety of ML29-SF vaccination and supporting the future evaluation of this LASV vaccine, including in clinical trials.

6218

FROM RESISTANCE SIGNAL TO NATIONAL ACTION: OPERATIONALIZING MULTIPLE FIRST-LINE THERAPIES TO ADDRESS ANTIMALARIAL DRUG RESISTANCE IN BUSIA AND BUIKWE DISTRICTS, UGANDA

Steven Kikomeko[1], Thomson Ngabirano[1], Fredrick Makumbi[2], Gerald Rukundo[3], Catherine Maiteki-Sebuguzi[3], Pamela Achii[3], Gladys Tetteh[4], Naoko Doi[5], Lolade Oseni[5], Rima Shretta[5]

[1]Jhpiego, Kampala, Uganda, [2]Makerere University School of Public Health, Kampala, Uganda, [3]Ministry of Health, Kampala, Uganda, [4]Jhpiego, Washington, DC, United States, [5]Jhpiego, Baltimore, MD, United States

Uganda remains among the world's highest malaria burden countries and has relied on artemether-lumefantrine (AL) as the single first-line treatment for uncomplicated malaria for over a decade. Therapeutic efficacy studies conducted in 2022-2023 documented declining AL efficacy below the WHO 90% threshold, while molecular analyses identified validated PfK13 mutations, including C469Y and A675V, associated with delayed parasite clearance. These findings signaled emerging antimalarial drug resistance (AMR) and underscored the need for coordinated national strategies to preserve ACT efficacy. Beginning in 2025, the Ministry of Health's National Malaria Elimination Division, in collaboration with the STOP-AMDR project led by Jhpiego and partners, initiated a phased implementation-readiness process to operationalize WHO-recommended Multiple First-Line Therapies. Activities focused on governance, evidence generation, policy alignment, and system readiness, including a kick-off meeting, co-creation workshop, multisectoral consultations, district entry meetings and community engagement, establishment of a Research Advisory Committee, and ethical approvals. National modelling informed sub-national ACT deployment strategies aligned to facility levels and consumption patterns, with Busia and Buikwe selected as pilot districts based on TES findings, malaria burden trends, and cross-border considerations. This enabled development of context-specific MFT deployment models aligned with Uganda's AMR strategy ahead of phased rollout. AMR mitigation training modules, job aids, and supervision tools were revised, and ACT quantification was integrated into national supply planning. Civil society engagement included mapping 52 CSOs and engaging 288 stakeholders to support demand generation, adherence, and ownership. Uganda's experience demonstrates how resistance surveillance evidence can be translated into coordinated national action, integrating policy alignment, community engagement, operational planning, and supply chain and system readiness to enable scalable MFT deployment and AMR mitigation.

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PERSISTENT PARASITEMIA AFTER ARTEMETHER LUMEFANTRINE TREATMENT: SIGNALS OF SUBOPTIMAL DOSE AND RECRUDESCENCE IN FIELD ISOLATES

Farid Salim Abdi[1], Agnes Chelangat Cheruiyot[2], Redemptah Yeda[2], Edwin Mwakio[2], Gladys Chebet Chemwor[2], Charles Okello[3], Dennis Wekesa Juma[2], Benjamin Opot[4], Raphael Onyango Okoth[2], Jackline Jumah[2], Risper Nyaboke Maisiba[2], Maurine Atieno Mwalo[2], Loius Powsang[5], Hoseah Akala[2]

[1]Walter Reed, KISUMU, Kenya, [2]Department of Emerging Infectious Diseases (DEID), Walter Reed Army Institute of Research (WRAIR-Africa, Kenya Medical Research Institute (KEMRI), Kisumu, Kenya, [3]Department of Emerging Infectious Diseases (DEID), Walter Reed Army Institute of Research (WRAIR-Africa, Kenya Medical Research Institute (KEMRI), KISUMU, Kenya, [4]Department of Emerging Infectious Diseases (DEID), Walter Reed Army Institute of Research (WRAIR-Africa, Kenya Medical Research Institute (KEMRI), Nairobi, Kenya, [5]Walter Reed Army Institute of Research-Africa, KISUMU, Kenya

Reports on widespread resistance on artemisinin and its derivatives in Southeast Asia and emerging partial artemisinin resistance in Africa

warrants surveillance for updating policies. This study aims to find out if there is a suboptimal response of *Plasmodium falciparum* to Artemether Lumefantrine (AL) by evaluating follow-up samples from individuals previously treated with AL. *Plasmodium falciparum*-positive samples collected from symptomatic individuals aged 6 months and above. 34 culture-adapted samples were tested for in vitro susceptibility to chloroquine (CQ), atovaquone (ATOV), Artemether (AT), dihydroartemisinin (DHA), amodiaquine (AQ), artesunate (AS), and mefloquine (MQ). Day -7 lumefantrine concentrations, and genotyping were done to assess parasite persistence for potential drug tolerance. A total of 1,283 whole blood samples were collected, of these; 34 samples were positive for *P. falciparum*. These include 11 day-7, 10 day-14, 5 day-28, 2 day-42 ,1 day-1, 1 day-2, 1 day-3, and 1 day-73. 6/34 samples successfully adapted and in vitro susceptibility testing against antimalarials showed median IC_{50} values (IQR, ng/mL) of CQ 27.67 (19.29-52.92), ATOV 9.910 (4.899-12.50), AT 16.43 (15.94-19.31), DHA 5.017 (3.485-7.578), AQ 4.916 (4.247-20.56.), AS 1.17 (0.79-1.33), and MQ 61.07 (37.75-111.2). Genotyping using MSP1, MSP2 and GLURP markers classified 4/6 isolates as recrudescent infections. Median drug IC_{50} comparison between day-0 and follow-up isolates using the Wilcoxon signed-rank test showed no significant difference. Lumefantrine concentration analysis indicated that 8 day-7 samples had levels higher than 260 ng/mL, suggesting persistent parasitemia despite adherence, while 3 day-7 samples had lower concentrations consistent with possible nonadherence. Most isolates remained susceptible to the tested antimalarials, with no significant differences between baseline and follow-up IC_{50} values. However, recrudescent infections and persistent parasitemia despite adequate lumefantrine exposure highlight the need for continued therapeutic efficacy monitoring and adherence surveillance.

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SURVEILLANCE OF COPY NUMBER VARIATION AND SINGLE NUCLEOTIDE POLYMORPHISMS IN *PLASMODIUM FALCIPARUM* ISOLATES FROM SELECTED COUNTIES IN KENYA DURING A PERIOD OF INCREASING *KELCH* 13 MUTATIONS

Risper Nyaboke Maisiba[1], Benjamin Opot[2], Raphael Onyango Okoth[3], Agnes Chelangat Cheruiyot[3], Redemptah Yeda[4], Farid Salim[1], Jackline Jumah[5], Maurine Mwalo[6], Edwin Mwakio[7], Charles Okello[1], Robert Clark Haynes[8], Dennis Wekesa Juma[9], Luis Powsang[1], Hoseah Akala[4]

[1]KEMRI-WALTER REED, Kisumu, Kenya, [2]United States Army Medical Research Unit - Kenya, Nairobi, Kenya, [3]HJFMRI, Kisumu, Kenya, [4]USAMRU-K, Kisumu, Kenya, [5]USAMRD-A/K-KEMRI, Kisumu, Kenya, [6]USAMRD/KEMRI, [7]Kenya Medical Research Institution, Kisumu, Kenya, [8]DPO, AE, United States, [9]United States Army Medical Research Directorate-Kenya, Kisumu, Kenya

Genomic aberrations such as copy number variations (CNV) and single nucleotide polymorphisms (SNP) are the primary drivers of malaria drug resistance. Robust surveillance systems that incorporate these markers are essential in determining antimalarial drug efficacy. This study determined the changes in copy number variation (CNV) of *Plasmodium falciparum multidrug resistance gene1* (*Pfmdr1*), single nucleotide polymorphisms (SNPs) in *Pfmdr1*, *Plasmodium falciparum chloroquine resistance transporter* (*Pfcrt*), and *Plasmodium falciparum K13* (*Pfk13*) genes. A total of 236 blood samples were collected from individuals presenting with malaria symptoms at selected health facilities. They were analyzed for CNV using real time PCR. A subset of these samples (n=154) was sequenced for polymorphisms in *Pfmdr1* codons N86Y/Y184F; *Pfcrt* codons 72-76, and codon *PfK13* by Sanger sequencing. A total of 32/154 (20.8%) samples had *Pfmdr1* multiple copies. Additionally, 72/154(46.8%) infections had *Pfmdr1* mutations at codon 184F. A subset 19/154(12.3%) had multiple copies with 6/154(3.9%) presenting both *Pfmdr1* (184F) and *Pfcrt*-CVIET mutations. Additionally, 53/154(34.4%) had single copies with, 10/154(6.5%) having *Pfmdr1* mutations at codon 184F */Pfcrt* -CVIET mutations. Up to 15/154 (9.7%) that had single copies and harbored Pfk*13* mutations at loci A675V 11/154(7.1%), A617P 2/154(1.3%), and S536P 1/154(0.6%). 5/154(3.2%) had P*fmdr1*184 F mutation. Site appears to impact CNV, *Pfmdr1* and *Pfcrt* SNPS with p=0.000. The presence of multiple copy numbers 32/154 (20.8%) in study samples points to a possible risk in reduced malaria treatment efficacy involving lumefantrine. The frequencies of parasites harboring *Pfcrt*-CVIET haplotype, alongside increased Pfmdr1 copy numbers and *184F haplotypes* warrant continued monitoring of ACTs as they may play a role in driving malaria resistance mechanisms. It is important to incorporate *Pfk13* mutations surveillance across all malaria epidemiological zones so as to strengthen malaria control at national as well as regional level thus enhancing health security.

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DESIGN, SYNTHESIS, ANTIMALARIAL SCREENING, AND ADMET PROFILING OF DERIVATIVES OF MMV902507

Fritz Egbert Plange, Egbert Plange, Deborah Annor, Shaban Mohammed, Akua Gyamfuaa Adu-Gyamfi, Magdalene Dzigbordi Togoh, Prof. Richard Amewu

Drug Innovation Group/ University of Ghana, Accra, Ghana

Malaria is a protozoan, parasitic disease transmitted to humans through the bites of infected female mosquitoes *Anopheles* mosquitoes. According to the 2025 World Malaria Report, nearly half the world's population lives in areas at risk of malaria transmission in 80 countries and territories. In 2025, malaria caused an estimated 282 million clinical episodes and 610,000 deaths. Despite the availability of treatment for malaria, drug resistance has hampered the utility of existing medicines. The identification of novel biochemical targets in *P. falciparum* has provided new opportunities for antimalarial drug development. MMV902507 chemical scaffolds were identified in phenotypic screening of the Charles River BioFocus and Bayer proprietary libraries as an antimalarial hit. The scaffold MMV902507 possessed antimalarial activity against the blood and liver stages of the malaria parasite. It displayed no cross-resistance against the drug resistant Dd2 strain of malaria. Here, we report the SAR exploration of MMV902507 via the synthesis of several derivatives based on the MMV902507 scaffold. Compounds FP046, FP087, and FP120 gave IC_{50} values of 0.046 µM, 0.062 µM, and 0.096 µM, respectively, which were superior to the 0.12 µM obtained for the re-synthesized hit when tested against the 3D7 strain. Evaluation of ADME properties revealed compounds with improved stability in rat and human microsomes. Compound FP069 retained *in vitro* antimalarial activity as MMV902507 but possessed superior clearance, half-life, and solubility. Assessment of potential cardiotoxicity risks showed no such issues with the series.

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OPERATIONALIZING MULTIPLE FIRST-LINE THERAPIES FOR MALARIA: IMPLEMENTATION RESEARCH FROM THE STOP-AMDR PROJECT IN DEMOCRATIC REPUBLIC OF CONGO, NIGERIA, RWANDA AND UGANDA

Mark M. Kabue[1], Rima Shretta[1], Gladys Tetteh[2], Lolade Oseni[2], Naoko Doi[1]

[1]Jhpiego, Johns Hopkins University affiliate, Baltimore, MD, United States, [2]Jhpiego, Johns Hopkins University affiliate, Washington DC, MD, United States

The effectiveness of artemisinin-based combination therapies (ACTs) in malaria case management is increasingly threatened by emerging partial artemisinin resistance, exacerbated by reliance on a single first-line regimen. Multiple first-line therapy (MFT), which deploys two or more ACTs simultaneously or in rotation, is a promising strategy to reduce drug pressure and extend therapeutic lifespan. However, critical evidence on how to implement MFT effectively, affordably, and at scale within routine health systems is lacking. A multi-country implementation research study that employs a convergent mixed-methods design with sequential pre-post cross-sectional assessments is underway in the Democratic Republic of Congo, Nigeria, Rwanda, and Uganda (2026-2028). Study sites were purposively selected in partnership with national malaria programs to capture heterogeneity transmission intensity and implementation

environments. Research questions assess: (1) feasibility and acceptability of MFT implementation; (2) policy, supply-chain, and health system requirements and enablers; and (3) costs, cost drivers, and affordability of sustainable scale-up. Data collection includes household, provider, and private sector surveys; key informant interviews with policymakers and program leaders; ACT procurement, deployment and use; and cost analyses. Baseline data collection begins in May 2026, with early findings shared by September 2026. Analysis of baseline ACT-disaggregated coverage will characterize antimalarial use from public and private sectors. Data on implementation bottlenecks, enablers, procurement and distribution of multiple ACTs, provider prescribing behavior, and community treatment-seeking patterns will be shared. Financial and economic costs across settings will be generated. Evidence generated will contribute to national policy/strategy decisions and global guidance on MFT, thereby accelerating the transition to optimized treatment strategies that delay antimalarial resistance.

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LEARNINGS FROM ACT QUANTIFICATION DURING EARLY STAGE OF MULTIPLE FIRST-LINE THERAPIES (MFT) SCALE-UP IN BURKINA FASO

Ragnagnewende Medard Aristide Nacoulma[1], Noufou Gansonre[2], Yacouba Belem[1], Ousmane Badolo[1], Sidzabda Christian Bernard Kompaore[2], Naoko Doi[3], Gladys Tetteh[3]

[1]Jhpiego, Ouagadougou, Burkina Faso, [2]SP/Palu, Ouagadougou, Burkina Faso, [3]Jhpiego, Baltimore, MD, United States

Artemisinin resistance threatens malaria control in sub-Saharan Africa, and Burkina Faso started scaling up MFTs in 2023. With no consumption data for the newer ACTs (ASPY) to inform quantification, the SP/Palu used an iterative approach to quantify newer ACTs. An iterative, six-stage approach was used to calibrate the quantification of the newer ACTs:1) Use of morbidity-based estimates; 2) Use of logistics data from the e-LMIS from health facilities with high reporting rates to monitor stock level and consumption;3) Roll-out of a weekly stock reporting tool (TLOH) for prevention of stock-outs; 4) Weekly and monthly logistics data review meetings to assess data quality and identify reporting gaps. 5) Quarterly supply plan revision using e-LMIS, DHIS2 and TLOH data to adjust orders; 6) Quarterly supportive supervisions to ensure appropriate prescription and reporting at the health facility level. The Central Medical stores received a larger quantity of ASPY than needed, creating a significant expiry risk across multiple batches. Three root causes were identified. First, the dose to age-band conversion errors: due to the lack of historical consumption data, a conversion factor derived from AL experience was used. Second, the dose to pack conversion errors systematically overestimated pack quantities procured. And thirdly, the poor and inconsistent reporting across facilities in the e-LMIS did not allow timely adjustment of orders. ACT quantification for MFT in Burkina Faso required three adaptations from the standard process: application of a treatment to pack conversion factor for ASPY informed by age-stratified consumption data; a lightweight consumption tracking tool deployed before e-LMIS (NetSigl) is operational; monthly data review and quarterly forecasting review comparing forecast against actual consumption to refine assumptions and adjust procurement. Together, these adaptations form an operationally feasible adaptive quantification cycle replicable by any country introducing a new ACT without prior consumption data.

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COMMODITY READINESS FOR INITIATING MFT IMPLEMENTATION: LESSONS FROM EARLY IMPLEMENTATION IN THE SIX STOP-AMDR PROJECT SUPPORTED COUNTRIES

Aristide Nacoulma[1], Naoko Doi[2], Gladys Tetteh[2], Rima Shretta[2]

[1]Jhpiego, Ouagadougou, Burkina Faso, [2]Jhpiego, Baltimore, MD, United States

The emergence of artemisinin partial resistance in sub-Saharan Africa has accelerated interest in deploying Multiple First-Line Therapies (MFT) as a resistance management strategy. Initiating the transition from a single first-line ACT to multiple first-line ACT requires careful planning and coordination to ensure multiple ACTs are available in a timely manner. The STOP-AMDR project is the first Unitaid-funded initiative supporting Burkina Faso, the DRC, Kenya, Nigeria, Rwanda, and Uganda to accelerate operational readiness required for MFT. We supported the stepwise approach to advance procurement and supply chain readiness to initiate MFT implementation. Key intervention included: 1) development of country-specific MFT plans 2) technical support for updating national malaria treatment guidelines to incorporate MFT; 3) forecasting for multiple ACTs 4) development of implementation roadmaps and supply plans; 5) development of stock visibility and pipeline tracking tools 6) funding and commodity gap analyses; 7) multi-global stakeholders advocacy and coordination with procurers, to secure uninterrupted commodity availability and 8) in-country malaria partner coordination led by NMPs to ensure aligned implementation timelines. Across the six countries, STOP-AMDR support enabled timely treatment guideline revisions, completion of initial MFT quantification exercises, and securing commodity commitments for pilot launch and scale up of MFT. Stock tracking tool improved supply pipeline monitoring for ease of advocacy and engagement with global donors. Coordinated multi-stakeholder engagement was critical in addressing financing gaps for higher-cost ACTs and aligning commodity availability with training schedules. Key policy and implementation accelerators include early NMP ownership, integrated forecasting aligned with existing in country supply and procurement systems and governance, and early planning for proactive donor engagement. The presentation will outline the key drivers, actors, and that accelerated the adoption and roll out in each unique context using a descriptive policy framework.

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GENOMIC SURVEILLANCE OF ANTIMALARIAL DRUG RESISTANCE IN MALI: SULFADOXINE-PYRIMETHAMINE RESISTANCE FIXATION AND EMERGING ARTEMISININ MARKERS

Abdoulaye Diawara[1], Hamadoun Garba[2], Mamadou Sanogo[3], Mahamadou Diakité[1], Jeffrey G. Shaffer[4]

[1]African Center for Excellence in Bioinformatics of Bamako (ACE-B), University of Sciences, Techniques and Technologies of Bamako (USTTB), Bamako, Mali., Bamako, Mali, [2]Universite Scientifique Libre de Bamako., Bamako, Mali, [3]Université Félix-Houphouët-Boigny Mali, Bamako, Mali, [4]Tulane University School of Public Health and Tropical Medicine, New Orleans, Louisiana, LA, United States

Background: Malaria remains the leading cause of morbidity and mortality in Mali, with drug-resistant *Plamodium falciparum* threatening recent control gains. Methods: We analyzed 2,887 clinical isolates collected between 2014 and 2022 from four epidemiologically distinct sites in Mali using targeted amplicon sequencing to characterize resistance markers in pfk13, pfcrt, pfmdr1, pfdhfr, pfdhps, and pfarps10. Results: The near-fixation of sulfadoxine-pyrimethamine resistance markers (pfdhfr IRNI + pfdhps AGEAA) reached 91% prevalence by 2022 and was associated with 2.3-fold higher treatment failure rates (95% CI: 1.7-3.1). The artemisinin resistance genetic background (pfarps10-V127M) was detected in 12.1% of isolates from Koila. Chloroquine resistance (pfcrt CVIET) persists with cyclical patterns (19-29% prevalence) despite drug withdrawal. Spatial analysis identified resistance hotspots: Sirakorola (pfmdr1 86N surged to 76.7%) and Dangassa (pfdhps 540K reached 94.8%). Conclusion: Multidrug-resistant *P. falciparum* is rapidly evolving and spreading in Mali. Urgent transition from SP-based preventive therapies, enhanced surveillance for emerging artemisinin resistance, and targeted containment in high-risk corridors are needed.

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IMPACT OF CULTURE ADAPTATION ON DRUG SUSCEPTIBILITY AND CLONAL DYNAMICS OF *PLASMODIUM FALCIPARUM* ISOLATES IN UGANDA

Stephen Orena[1], Martin Okitwi[2], Oriana Kreutzfeld[3], Thomas Katairo[1], Jennifer Lagac[3], Jackson Asiimwe[1], Patrick Angutoko[1], Bersabeh Tafesse[3], Solomon Opio[1], Evans Muhanguzi[1], Emmanuel Data[1], Oswald Byaruhanga[1], Innocent Tibagambirwa[1], Moses R. Kamya[1], Melissa Conrad[4], Samuel Lubwama Nsobya[1], Philip Rosenthal[5]

[1]Infectious Diseases Research Collaboration, Kampala, Uganda, [2]Infectious Diseases Research Collaboration, Uganda, Kampala, Uganda, [3]University of Calfornia San Francisco, San Francisco, CA, United States, [4]John Hopkins School of Public Health, Baltimore, MD, United States, [5]Univ of California San Francisco, Mill Valley, CA, United States

Plasmodium falciparum infections in high transmission settings are usually polyclonal, with the potential for varied levels of drug susceptibility and fitness among infecting clones. During culture-adaptation, clones with better growth fitness may dominate, impacting results based on studies of culture-adapted parasites. To assess the impacts of culture-adaptation, for 90 *P. falciparum* isolates collected from northern and eastern Uganda in 2024-2026, we are comparing drug susceptibilities and genotypes immediately after sample collection and after culture-adaptation. Susceptibilities for 7 antimalarial compounds were determined using a 72-hour growth inhibition (IC_{50}) assay with SYBR Green detection. Genotyping will be performed using the Multiplexed Amplicons for Drugs, Diagnostics, Diversity, and Differentiation using High-Throughput Targeted Resequencing (MAD^{4}HatTeR) panel. The mean number of days for culture adaptation was 37 days. Drug susceptibilities before and after culture-adaptation were similar for lumefantrine (median IC_{50} 14.6 nM vs 13.7 nM) and mefloquine (18.4 nM vs 18.8 nM), but susceptibilities decreased for dihydroartemisinin (median IC_{50} 3.3 nM vs 4.0 nM) and the aminoquinolines chloroquine (11.0 nM vs 18.7 nM), monodesethylamodiaquine (8.1 nM vs 10.1 nM), piperaquine (4.6 nM vs 6.2 nM), and pyronaridine (0.88 nM vs 2.3 nM). For some individual isolates, susceptibilities for certain drugs changed by 2-fold or more after culture adaptation. Comparison of genotypes of these isolates before and after culture adaptation is pending. Selection of clones with altered susceptibilities during culture adaptation may have been due to fitness constraints. Importantly, genotypes and phenotypes observed in culture adapted parasites may not fully reflect the features of clinical isolates.

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PREVALENCE OF ANTIMALARIAL DRUG RESISTANCE MARKERS IN SYMPTOMATIC AND ASYMPTOMATIC MALARIA INFECTIONS IN SELECTED REGIONS OF MAINLAND TANZANIA

Dativa Pereus[1], Hussein J. Semboja[2], Rule Budodo[3], Misago Dimson Seth[4], Rashid Ally Madebe[4], Catherine Bakari[3], Salehe Mandai[3], Angelina J. Kisambale[3], Gervas Chacha[2], Doris David Mbata[5], Daniel Protasy Challe[6], Daniel Anderson Petro[7], Solomon Mickson Mwakasungula[8], Happyness A. Mwailunga[3], Laurencia S. Tangawizi[3], Gideon M. Isaya[3], Ninja S. Bisaga[2], Baraka W. Mbuba[3], Celine Isaack Mandara[5], Daniel Baraka Mbwambo[9], Sijenunu Aaron[10], Abdallah Lusasi[11], Samwel Lazaro[10], Stella Kajange[12], Jeffrey A. Bailey[13], Jonathan J. Juliano[14], Victor Atunga Mobegi[15], Gerald Juma[16], Deusdedith R.S. Ishengoma[3]

[1]Muhimbili University of Health and Allied Sciences, Dar Es Salaam, United Republic of Tanzania, [2]National Institute for Medical Research, Tanzania, Dar Es Salaam, United Republic of Tanzania, [3]Ifakara Health Institute, Dar Es Salaam, United Republic of Tanzania, [4]National Institute for Medical Research, Tanzania, Dar es Salaam, United Republic of Tanzania, [5]National Institute for Medical Research, Dar Es Salaam, United Republic of Tanzania, [6]National Institute for Medical Research, Tanga, United Republic of Tanzania, [7]United Republic of Tanzania, [8]Ifakara Health Institute, Bagamoyo, United Republic of Tanzania, [9]National Malaria Control Program-Tanzania, Dodoma, United Republic of Tanzania, [10]National Malaria Control Program, Dodoma, United Republic of Tanzania, [11]Dar Es Salaam, United Republic of Tanzania, [12]Prime Minister's Office, Regional Administration and Local Government, Dodoma, United Republic of Tanzania, [13]Brown University, Providence, RI, United States, [14]Univ of North Carolina, Chapel Hill, NC, United States, [15]The University of Nairobi, Nairobi, Kenya, [16]University of Nairobi, Nairobi, Kenya

Asymptomatic *Plasmodium falciparum* infections often create ecological conditions that allow resistance alleles to circulate and persist undetected in the absence of active surveillance. This study assessed the prevalence of antimalarial drug resistance markers in *P. falciparum* from symptomatic patients attending health facilities (HF) and asymptomatic individuals sampled in the community (CSS) surveys in five regions of Mainland Tanzania. Dried blood spot samples were collected from 6,113 individuals (2,824 symptomatic and 3,289 asymptomatic individuals) from January to August 2023. DNA was extracted using the Tween-Chelex method; sequencing was performed using the molecular inversion probes assay. Data analysis was performed using MIPtools. Of the tested samples (n=6,113), 4,335 (80%) were successfully genotyped. In the HF survey, the weighted average regional prevalence of k13 R561H was 7.4% in Kagera and 3.5% in Kigoma. Other k13 mutations (A675V, P441L, R622I) were present at low prevalence, though R622I reached 7.9% in Ruvuma (in the HF survey) and 16.7% in Kigoma (in the CSS). The antifolate resistance markers (dhfr: N51I, C59R, and S108N; dhps: A437G and K540E) were nearly fixed, the rare dhfr I164L reached 13.7% in Kagera in the HF survey. The dhps A581G prevalence ranged from 51.5% in Tanga in the HF survey to 66.7% in Kigoma in the CSS. The pfcrt K76T mutation was detected at moderate prevalence, ranging from 6.3% in Kigoma in the HF survey to 25.0% in Kigoma in the CSS. Partner drug resistance mutations remained low, except for mdr1 Y184F, which ranged from 38.0% in Kigoma in the HF to 100.0% in Njombe in the CSS survey. These findings indicate localized emergence of artemisinin partial resistance in northwestern Tanzania, with sporadic occurrence of other k13 mutations in other regions. Mutations in asymptomatic infections reveal a hidden reservoir sustaining transmission and potential ART-R spread in Tanzania. High antifolate resistance (dhfr I164L, dhps A581G) reflects ongoing SP pressure. Moderate pfcrt K76T and limited partner drug resistance support continued ACT efficacy, though surveillance remains essential.

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OPTIMIZING MALARIA CASE MANAGEMENT IN NIGERIA: EVIDENCE OF ACT DEPLOYMENT PATTERNS IN ENUGU STATE

Charity Ifeyinwa Anoke[1], Bright C. C. Orji[2], Nnenna Ogbulafor[3], Emmanuel David Shekarau[4], Simon Ijezie[1], Martin Meremikwu[5], Olugbenga Ayodeji MOKUOLU[6], George Ugwu[7], Adetiloye Oniyire[1], Ifeyinwa Ani-Osheku[8], Uchechukwu Okenwa[7], Ifeoma Otiji[9], Mubarak Badmasi[1], Lolade Oseni[10], Mark Kabue[11], Joseph Enne[1]

[1]Jhpiego, Abuja, Nigeria, [2]Jhpiego, Wuye, Abuja, Nigeria, [3]Management Sciences for Health, Abuja, Nigeria, [4]National Malaria Elimination Programme, Federal Ministry of Health, Abuja, Abuja, Nigeria, [5]Univ of Calabar Teaching Hosp, Calabar, Cross River State, Nigeria, [6]University Of Ilorin, Ilorin, KW, Nigeria, [7]Ministry of Health, Enugu, Nigeria, [8]Primary Health Development Agency, Enugu, Nigeria, [9]State Malaria Elimination Programme, Enugu, Nigeria, [10]Jhpiego, Baltimore, MD, United States, [11]Jhpiego, Johns Hopkins University affiliate, Baltimore, MD, United States

Nigeria adopted the Test, Treat, Track (3T) strategy in 2010 and in 2020 expanded guidelines to liberalize artemisinin-based combination therapies (ACTs) as first-line treatment. While policy improved access to multiple ACTs, its effect on reducing pressure on any one drug is unassessed. This paper reviews Nigeria's ACT diversification against WHO guidance to reduce selective pressure and delay resistance. A synthesis review and retrospective extraction of ACT consumption (Dec 2024-Oct 2025) was conducted in 45 purposively selected facilities across three Enugu LGAs. Kobo-based data from outpatient registers, EMRs, and consumption records captured age, diagnosis, confirmed cases, and ACT dispensed for stratified analysis of malaria incidence and ACT use. Findings show that Enugu State shifted from the use of two ACTs (AL, AA) prior to

2024 to four ACTs (AL, AA, ASPY, DP) in 2024, reflecting compliance with national guidelines. However, irrational ACT use, and high level of presumptive treatment (15,362 confirmed cases; 31,367 ACT dispensed, i.e, 49% of 31,367 persisted, contradicting the 3Ts approach. ACT market share shifted from 73% AL and 27% AA to 54% AL, 15% AA, 19% DP, 12% ASPY, reducing pressure on any single drug, but lacking structured allocation strategy. The distribution largely aligns to WHO recommended distribution thresholds for deploying 4 drugs (≥35% for two ACTs, ≥25% for three ACTs, and ≥15% for four ACTs) except ASPY at 12% below suggested 15%. Despite diversification progress, ACT deployment showed gaps in quantification, adherence, and prescribers' knowledge affecting supply chain management, guidance, and monitoring. This gap between guideline intent and WHO MFT recommendations highlights the need for clear national policy, enhanced molecular surveillance, and integration of resistance data. Greater efforts are needed to ensure constant availability of test kits to reduce presumptive treatment. A structured ACT deployment model per WHO MFT guidelines should replace liberal use, reduce drug pressure, and ensure confirmed diagnosis before treatment, supporting a scalable resistance mitigating strategy.

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NOVEL GLUCOSE-6-PHOSPHATE DEHYDROGENASE VARIANTS CONTRIBUTE TO HIGH GENETIC DIVERSITY IN AN AFRO-COLOMBIAN POPULATION EXPOSED TO VIVAX MALARIA

Lina Zuluaga-Idarraga[1], Alexandra Rios-Orrego[1], Juan Esteban Martinez-Lopez[1], Tatiana Maria Lopera-Mesa[1], Daniel Aguirre-Acevedo[1], Mateo Castañeda-Pabón[1], Omer Campo-Nieto[2], Gustavo Gamez[1], Jaclyn Flewelling[3], Lawrence M. Barat[3], Michelle S. Hsiang[4], Leopoldo Villegas[3]

[1]University of Antioquia, Medellin, Colombia, [2]University of Antioquia, MEDELLIN, Colombia, [3]U.S. President's Malaria Initiative (PMI) Impact Malaria project, Washington D.C., WA, United States, [4]University of California San Francisco, San Francisco, CA, United States

Glucose-6-phosphate dehydrogenase deficiency (G6PDd) limits safe use of 8-aminoquinolines for radical cure of *Plasmodium vivax* (Pv). The frequency of G6PD single-nucleotide variants (SNVs) associated with hemolysis has been well described in Africa and Asia, while data from Latin America remain scarce. This study aimed to identify SNVs in the coding regions of the *G6PD* gene in Quibdó, Chocó, Colombia, a municipality with high Pv endemicity and predominantly Afro-Colombian population (91%). In a cross-sectional survey of 2003 participants, quantitative G6PD activity was measured using the Standard Biosensor test. For all participants found to be G6PD deficient (85 men; 84 women) or intermediate (143 women), and a random sample of G6PD normal (59 men; 61 women) participants, SNVs were analyzed across the 13 exons by a custom-designed panel of next-generation sequencing (Illumina), with a subset of identified variants further confirmed by traditional Sanger sequencing. 284 distinct SNVs were identified. At least one variant was found in 68% of deficient individuals, 54.3% of intermediates, and 22.3% of those with normal activity. The most frequent clinically known variants were A+ (4.7%), A– (4.4%), Farroupilha (1.9%), Chatham (1.6%), Yucatan (1.6%), Seoul (1.4%), and Ilesha (1.1%). Novel variants Quibdó1 and Quibdó2 were detected in 4.2% and 2.3%, respectively. Variants A+, A–, Farroupilha, Chatham, and Quibdó1 were more frequent among deficient individuals compared to those with normal activity (10.4% vs. 1.8%; 11.4% vs. 7.3%; 5.4% vs. 0%; 4.5% vs. 0%; and 11.9% vs. 4.2%, respectively). Only 42.6% of the deficient individuals who carry variants had only one of those, while the majority harbored multiple variants. Prior studies of G6PDd in Latin America have focused primarily on the A+, A-, and Mediterranean variants; our findings that other, novel variants partially explain G6PDd in an Afro-Colombian population offer an important advance, informing future studies of their functional impact and guiding the implementation of new *P. vivax* radical cure treatment.

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QUANTIFYING FOR NEW MULTIPLE FIRST-LINE THERAPIES (MFT) ACTS AND RELATED RESOURCE MOBILIZATION IN READINESS FOR SUSTAINED AVAILABILITY AND SUCCESSFUL MFT IMPLEMENTATION IN KENYA

Ndinda Kusu[1], Sammy Mahugu[2], Emmah Nyandigisi[2], Edwin Onyango[2], Meera Shah[3], Morris Kiio[1], Paul Nyachae[1], George Wadegu[1], Aristide Nacoulma[4], Gladys Tetteh[4], Rima Shretta[4]

[1]Jhpiego, Nairobi, Kenya, [2]Ministry of Health, National Malaria Control Program, Nairobi, Kenya, [3]Chemonics Global Health Supply Chain Program, Nairobi, Kenya, [4]Jhpiego, Baltimore, MD, United States

In 2023, Kenya reported 5.5 million malaria cases and 769 deaths. The country is transitioning to a Multiple First-Line Therapies (MFT) strategy using rotational Artemisinin-based Combination Therapies (ACTs) every two years to reduce drug pressure, prolong efficacy, and address emerging resistance. Forecasting and quantification (F&Q) for MFTs is complex due to limited historical data and higher costs of newer ACTs, necessitating accurate planning and sustainable financing. In November 2025, the Ministry of Health (MOH), National Malaria Control Program, and partners supported F&Q for malaria commodities, including new ACTs in Kenya's first MFT implementation plan. Stakeholders from the procurement and supply management committee integrated multiple data sources, including consumption trends, malaria morbidity, existing Artemether-Lumefantrine (AL) stocks, and population projections. Using standardized tools and pipeline analysis, F&Q incorporated regimen allocation ratios, and facility-level consumption. Resource mobilization included costed forecasts, financing gap analysis, and engagement the Government of Kenya, U.S. Government, and Global Fund. Forecasting aligned regimen with specific demand, minimizing risks of stockouts and expiries. Due to high AL stock levels, Kenya cancelled pending AL procurements, and funds were reprogrammed to support MFT procurement. MFT implementation will begin in October 2026 in four counties, allowing continued AL use in others. Ongoing stock status, pipeline, and expiry monitoring will support the transition. The estimated budget for the first rotation is $7.5 million, with Artesunate-pyronaridine as the key regimen. The Government and other partners will support the budget. Successful MFT implementation requires robust supply chains, strong F&Q, data systems, clear guidelines, continuous stock status, pipeline monitoring, and proactive financing. Kenya's approach provides offers a replicable framework for MFT quantification and resource mobilization applicable to other countries planning ACT diversification.

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INTEGRATING GENOMIC SURVEILLANCE INTO ROUTINE MALARIA SYSTEMS IN CAMEROON: INSIGHTS ON PARASITE DIVERSITY AND DRUG RESISTANCE

René Ghislain Essomba[1], Abel Lissom[1], Valentina Josiane Ngo Bitoungui[1], Victoria Simpson[2], Alfred A. Ngwa[3], Shavanthi Rajatileka[4], Hadja Hamsatou[1]

[1]National Public Health Laboratory, Yaoundé, Cameroon, [2]Welcome sanger institute, London, United Kingdom, [3]Medical Research Council Unit (MRC) The Gambia, London School of Hygiene & Tropical Medicine, Banjul, Gambia, [4]Welcome Sanger Institute, London, United Kingdom

Malaria remains a major public health burden in Cameroon, largely driven by *Plasmodium falciparum*. The continued efficacy of artemisinin-based combination therapies (ACTs), the cornerstone of malaria treatment, is threatened by the emergence and spread of drug-resistant parasite strains. A comprehensive and prospective genomic surveillance strategy is needed to generate timely, high-resolution data to inform national drug policies. This study evaluated the feasibility of integrating genomic surveillance into routine malaria systems in Cameroon and characterized parasite populations across diverse transmission settings. A prospective genomic surveillance study was conducted across 29 sentinel health facilities in three regions (Centre, Adamawa, and Far North) between March and September 2025. Blood samples from suspected malaria cases were

tested using microscopy and rapid diagnostic tests. DNA from confirmed *P. falciparum* infections was extracted and sequenced using an amplicon-based approach. Bioinformatics analyses were performed on validated sequences. Of 567 sequenced samples, 470 were included in the analysis. *P. falciparum* accounted for 98.5% of infections, with all four human Plasmodium species detected. Multiple-strain infections were observed in 42% of cases, decreasing from Centre (48.9%) to Far North (24.2%). ACTs showed 100% predicted efficacy. Chloroquine resistance was low (5.3%), increasing from Centre to Far North. In contrast, resistance to sulfadoxine (81.1%) and pyrimethamine (98.3%) was high, particularly among children under five years.These findings demonstrate the feasibility of integrating genomic surveillance into routine malaria systems in Cameroon. The high prevalence of sulfadoxine-pyrimethamine resistance may compromise chemoprevention strategies, while sustained ACT efficacy and emerging chloroquine sensitivity provide important insights for malaria control policy.

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ARE TOLERABILITY CONCERNS LIMITING ARTESUNATE-AMODIAQUINE USE? EVIDENCE TO INFORM MULTIPLE FIRST-LINE THERAPY STRATEGIES IN SUB-SAHARAN AFRICA

Shelby Cash[1], Leah Moriarty[2], Mateusz Plucinski[3], Tyler Johnson[1], Jessica Butts[4], Irene Cavros[5], Peter Troell[2]

[1]Centers for Disease Control & Prevention, Malaria Branch, Atlanta, GA, United States, [2]Centers for Disease Control and Prevention, Atlanta, GA, United States, [3]CDC, Atlanta, GA, United States, [4]Centers for Disease Control & Prev, Atlanta, GA, United States, [5]U.S. Centers for Disease Control and Prevention (CDC), Atlanta, GA, United States

Sustained reliance on artemether-lumefantrine (AL) across sub-Saharan Africa (SSA) has contributed to increased drug pressure and is associated with declining treatment efficacy, prompting interest in diversifying malaria treatment through multiple first-line therapy (MFT) strategies. Artesunate-amodiaquine (ASAQ) is a potential candidate for MFT, but concerns regarding tolerability and adherence may limit its use. To evaluate whether these concerns are supported by evidence, we conducted an integrated evidence synthesis assessing the ASAQ policy landscape across 47 SSA countries and Zanzibar, comparative safety and efficacy relative to AL using therapeutic efficacy studies across all age groups published from 2015-2024, and factors influencing ASAQ adherence from literature published between 2005-2025. Eighteen of 47 countries and Zanzibar (39.1%) included ASAQ as a first- or co-first-line therapy vs 39 (84.8%) for AL. In a patient-level analysis of 36 therapeutic efficacy studies from 24 countries (n=8,995 ASAQ; n=7,011 AL), serious adverse events were rare (<1%) and did not differ significantly between regimens. Non-serious adverse events, primarily gastrointestinal in nature, were reported more frequently with ASAQ than AL (21% vs 13% p<.05) although absolute differences were modest. Evidence from 29 studies on factors influencing adherence indicated that while ASAQ is perceived as less tolerable than AL, positive adherence behaviors, defined as documented completion of full treatment course as prescribed, are primarily driven by absence of side effects (e.g., dizziness, fatigue), clear provider instructions on dosing and side-effect management; and higher socioeconomic status. No studies in this synthesis investigated whether factors influencing adherence differed significantly between ASAQ, AL, or other antimalarials. These findings suggest that common anecdotal concerns regarding ASAQ tolerability, and its subsequent influence on adherence, may not be supported by available adverse event evidence, addressing a key barrier to its consideration as part of a MFT strategy to mitigate AL resistance.

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ANTIMALARIAL RESISTANCE GENES IN CEREBRAL AND UNCOMPLICATED MALARIA: A COMPARATIVE STUDY IN GHANA

Andrews Osei Obese[1], Elaine Asiwome Boadu[2], Rhoda Yeboah[3], Caleb Kobina Danso-Coffie[2], Emmanuel Boafo[4], Joanita Asirifi Yeboah[2], Ben Gyan[5], Linda Eva Amoah[6], Daniel Oduro[7]

[1]Ghana Infectious Disease Center (GIDC), Ashongman Estate, Ghana, [2]Accra, AA, Ghana, [3]Rhoda Yeboah, Accra, AA, Ghana, [4]Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana, Legon, Ghana, [5]Noguchi Memorial Inst for Med Res, Legon, Ghana, [6]Noguchi Memorial Institute for Medical Research (NMIMR), Accra, Ghana, [7]UNIVERSITY OF GHANA, Accra, AA, Ghana

Malaria continues to be a major public health threat to Sub-Saharan Africa. Although the use of effective antimalarials for treatment has improved malaria control and elimination, malaria continues to affect people in Africa. This is partly due to parasites developing resistance to currently used antimalarial drugs (failed treatment). Failed treatment could lead to increased morbidity and mortality as it permits complicated malaria (e.g. Cerebral malaria) development through the growth and multiplication of circulating parasites. This study sought to assess the association between antimalarial drug resistance genes in *Plasmodium falciparum* parasites and cerebral (severe) malaria development. The study used a total of 90 archived dried blood spots (DBS) samples from children, consisting of 45 cerebral malaria (CM) and 45 uncomplicated malaria (UM) cases. Nucleic acid (DNA) was extracted and analysed for the presence of parasites, and antimalarial resistant genes. Antimalarial resistant gene data was not successful for all samples tested. A total of seven different antimalarial resistant genes (Pfkelch 13, Pfcrt A220 & K76T, Pfmdr1 Y184F & N86Y, Pfdhfr N51I and Pfdhps A437G) were tested for in the samples from both CM and UM groups. A relatively higher proportion of these genes were seen in samples from CM than UM. Mutations were also detected in Pfkelch13 gene although these SNPs are not reported to confer drug resistance to *P. falciparum* parasites. Despite the high frequency of antimalarial drug-resistant genes in CM than UM groups, no significant association was found between CM and the presence of antimalarial drug-resistant genes. These findings may serve as a baseline data for further studies involving larger sample size, to inform policy making and implementation on malaria diagnosis, management, and elimination in Ghana.

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EVALUATING TAFENOQUINE-BASED COMBINATIONS TO IMPROVE RADICAL CURE OF *PLASMODIUM VIVAX* ON THE THAI-MYANMAR BORDER

Michele D. Spring[1], Sidhartha Chaudhury[2], Eric Garges[2], Krit Harncharoenkul[2], Edgie-Mark Co[2], Panupong Kowsurat[3], Chanasorn Srichan[4], Brian Vesely[2], Pattaraporn Vanachayangkul[2], Paphavee Lertsethtakarn-Ketwalha[2], Worachet Kuntawunginn[2], Sabaithip Sriwichai[2], Saowaluk Wongarunkochakorn[2], Montri Arsanok[2], Krisada Jongsakul[2]

[1]State University of New York, Upstate Medical University, Syracuse, NY, United States, [2]Walter Reed Army Institute of Research - Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [3]Department of Disease Control, Nonthaburi, Thailand, [4]Tha Song Yang Hospital, Tha Song Yang, Thailand

Plasmodium vivax is distinguished by its dormant liver-stage parasite (hypnozoite), which can cause malaria relapses months to years after initial treatment unless eradicated with an 8-aminoquinoline (8-AQ), such as primaquine (PQ) or tafenoquine (TQ). In late 2020, a clinical trial in Indonesia (INSPECTOR) evaluating TQ in combination with the blood-stage therapy dihydroartemisinin-piperaquine (DHA-PPQ) reported a high rate of TQ failure, with 79% of participants experiencing recurrent *P. vivax* infections. The mechanisms underlying these failures remain unclear, with proposed explanations including variation in hepatic hypnozoite burden, suboptimal fixed dosing of TQ (300 mg) compared with weight-based regimens, and potential drug-drug interactions between artemisinin-based therapies and

TQ. More recently, the EFFORT trial compared the efficacy of TQ combined with various artemisinin-based combination therapies (ACTs) against the standard regimen of chloroquine (CQ) plus TQ. Failure rates of DHA-PPQ plus TQ in Indonesia were not as high as observed in INSPECTOR (22.4% versus 79%) but still were higher than with CQ/TQ (14% in Ethiopia) and artesunate-pyronaridine plus TQ (15.6% in Cambodia), suggesting variability in partner drug performance. To further investigate the efficacy of TQ in combination with artemisinin derivatives, an ongoing clinical trial in western Thailand is evaluating TQ with artemether-lumefantrine (AL), a related ACT. The study also includes a delayed TQ administration arm, in which DHA-PPQ is used to clear parasitemia followed by TQ on Day 4, to assess potential negative drug-drug interactions. Both arms will be compared with CQ/TQ and DHA-PPQ/TQ regimens. The target enrollment is 50 glucose 6-phosphate dehydrogenase (G6PD)-normal participants per arm. Interim analyses of efficacy and safety outcomes will be presented.

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PHYTOCHEMICAL AND ANTIMALARIAL ACTIVITIES OF *OZOROA OBOVATA* (OLIV) R. FERN. AND A. FERN: A PLANT USED TO TREAT MALARIA IN BURKINA FASO

Aboubakar Soma

Institut de Recherche en Sciences de la Sante (IRSS), Ouagadougou, Burkina Faso

Phytotherapy is widely used to treat pathologies affecting the population of sub-Saharan Africa, particularly in Burkina Faso. This study aimed to evaluate the antiplasmodial activities of Ozoroa obo-vata extracts in vitro and in vivo. This plant is used to treat malaria in Burkina Faso. Aqueous and hydroethanolic leaf extracts were prepared. The macerates were freeze-dried, after which TLC analysis was used to determine the phytochemical compounds present. In vitro antiplasmodial activity was evaluated using the pLDH technique to determine the 50% inhibitory concentration. The Peter's four-day test was used to determine the percentage reduction in parasitaemia. TLC analysis revealed the presence of flavonoids, sterols, triterpenes and tannins in both extracts. The respective concentrations of total phenolics, flavonoids, and saponins were 352.27 ± 0.29 (mg EAG/g), 37.73 ± 0.47 (mg EQ/g), and 31.95 ± 1.71 (mg ED/g) in the aqueous extract and 266.71 ± 0.06 (mg EAG/g), 85.70 ± 3.76 (mg EQ/g), and 21.43 ± 0.055 (mg ED/g) in the hydroethanolic extract. The IC_{50} of both extracts was under 100 µg/ml against *Plasmodium falciparum* strains. The hydroethanolic extract showed suppression of 65.00% and 83.75% at 250 mg/kg and 500 mg/kg body weight, respectively. The aqueous extract reduced parasitaemia by 54.00% and 92.00% at 250 mg/kg and 500 mg/kg, respectively. The ability of the aqueous and hydroethanolic extracts of *Ozoroa obovata* to inhibit beta-haematin formation is low. The in vitro activity of the extracts against *P. falciparum* strains showed a moderately weak IC_{50}. O. obovata extracts exhibited good in vivo anti-plasmodial activity in NMRI mice at doses of 250 and 500 mg/kg of body weight for both the aqueous and hydroethanolic extracts.

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OPTIMIZATION OF GAMETOCYTE CONCENTRATION FOR *PLAMODIUM VIVAX* SPOROZOITE PRODUCTION

Shanan Immel[1], Holly Torano[1], John Woodford[2], Joel Goldberg[1], Jillian Neal[1], Junhui Duan[1], Heather Goodman[1], Soha Usmani[1], Juliana Sa[1], James McCarthy[3], Sumana Chakravarty[4], Asha Patil[4], B. Kim Lee Sim[4], Stephen L. Hoffman[4], Jen Hume[1], Patrick Duffy[1]

[1]NIH/NIAID, Bethesda, MD, United States, [2]QIMR Berghofer, Brisbane, Australia, [3]University of Melbourne, Melbourne, Australia, [4]Sanaria Inc, Rockville, MD, United States

Plasmodium vivax is the most geographically widespread human malaria parasite species, primarily affecting people in Asia and Central and South America. In 2025, LMIV initiated a *P. vivax* controlled human malaria infection (CHMI) study at the NIH Clinical Center using *P. vivax*-infected erythrocytes. We developed a protocol to concentrate *P. vivax* gametocytes from infected blood of the CHMI participants and then tested transmission to laboratory-reared mosquitoes using membrane feeding assays (MFA). Our protocol employs leukodepletion and Percoll gradient centrifugation to enrich gametocytes. To date, we have applied this technique to blood samples from 12 CHMI clinical trial participants across multiple time points post-inoculation and we are planning additional inoculations in 2026. Gametocyte enrichment significantly increased parasite signal by qPCR (18S and Pvs25) and enhanced mosquito infection prevalence and intensity in direct membrane feeding assays. Peak transmission occurred on day 11 post-inoculation. Importantly, concentrating gametocytes led to significantly higher salivary gland *P. vivax* (Pv) sporozoite (SPZ) yields from infected mosquitoes. This increase is critical for producing the large numbers of PvSPZ needed to help accelerate vaccine development. With a view to development of a sporozoite product fit for human use, we are adapting our process to use cGMP-compliant consumables with preliminary studies comparing Percoll with sterile Ficoll for the enrichment step. This optimized process could provide a reproducible system to study *P. vivax* transmission biology, evaluate serum functional transmission-blocking activity, and enable scalable production of PvSPZ for future studies.

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MOSQUITO TRANSMISSION DYNAMICS OF *PLASMODIUM VIVAX* INDUCED BLOOD STAGE MODEL IN US NAÏVE STUDY PARTICIPANTS

Jen CC Hume[1], Heather A. Goodman[1], Joel Goldberg[1], Shanan Immel[1], John Woodford[1], Julia Zemskova[1], Holly M. Torano[1], Soha Usmani[1], Emily Higbee[1], James McCarthy[2], Patrick E. Duffy[1]

[1]NIH/NIAID, Bethesda, MD, United States, [2]University of Melbourne, Melbourne, Australia

Controlled human malaria infections (CHMI) are increasingly used in early phase clinical trials to test the efficacy of investigational vaccines and novel antimalarial compounds. To date *Plasmodium falciparum* (Pf) CHMI studies are far more common due to its global health importance, the ease of *in vitro* parasite propagation, and recent development of cGMP manufactured cryopreserved Pf sporozoites suitable for human inoculation. However, *Plasmodium vivax* (Pv) studies have recently advanced utilizing the induced blood stage model of infection where cryopreserved asexual parasites are inoculated intravenously to initiate PvCHMI in study participants. Development of parasitemia and gametocytemia is monitored closely by qPCR and, along with clinical symptoms, used to guide the timing of transmission experiments with the goal of developing a Pv transmission model for testing transmission blocking vaccines. Here we present a summary of mosquito feeding studies conducted in the United States on approximately eighteen PvCHMI study subjects using the *P. vivax* isolate HMP013 (up to five feeds conducted per participant over multiple days). We compare mosquito infection rates between three different feeding assay types: 1. direct skin feeding (DSF) bioassay, 2. whole blood direct membrane feeding assay (WB-DMFA) and 3. gametocyte concentrated direct membrane feeding assay (conc-DMFA). We will discuss how transmission dynamics change over the course of PvCHMI infection and the optimal timing to maximize transmission endpoints. Transmission rates will be compared along with the advantages and disadvantages of the different assay types.

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PROBABILISTIC ESTIMATION OF TREATMENT EFFICACY IN *PLASMODIUM VIVAX* MALARIA

Emily S. Groves[1], James A. Watson[1], Aimee R. Taylor[2], Megha Rajasekhar[3], Tesfay Abreha[4], Ashenafi Assefa[5], Tamiru S. Degaga[6], Kian Soon Hoon[6], Jimee Hwang[7], Ashley Osborne[6], Anjana Rai[6], Julie A. Simpson[3], Edwin Sutanto[6], Dagimawie Tadesse[8], Walter R. J. Taylor[9], Kamala Thriemer[6], Hidayat Trimarsanto[6], Sarah Auburn[6], Ric N. Price[6], Robert J. Commons[6]

[1]University of Oxford, Oxford, United Kingdom, [2]Institut Pasteur, Paris, France, [3]University of Melbourne, Melbourne, Australia, [4]Columbia University, Addis Ababa, Ethiopia, [5]Ethiopian Public Health Institute, Addis Ababa, Ethiopia, [6]Menzies School of Health Research and Charles Darwin

University, Darwin, Australia, [7]Centers for Disease Control and Prevention, Atlanta, GA, United States, [8]Arba Minch University, Arba Minch, Ethiopia, [9]Mahidol University, Bangkok, Thailand

Plasmodium vivax malaria drug efficacy studies are confounded by an inability to differentiate between recurrences caused by treatment failures (recrudescences or relapses) and reinfections. Probabilistic modelling of time-to-recurrence and parasite genetic data can be used to estimate recurrence aetiology and improve the interpretation of *P. vivax* clinical trials. We analysed data from patients with *P. vivax* malaria enrolled into two randomised trials in Ethiopia. Patients were treated with a schizonticide with or without primaquine and followed for 12 months. Parasites at enrolment and recurrence were genotyped using a 93-microhaplotype panel. We developed a Bayesian population mixture model to estimate the probability of treatment failure per *P. vivax* recurrence based on its timing. We combined these prior estimates with parasite genetic data using a Bayesian genetic model (Pv3Rs) to estimate the final probability of treatment failure for each recurrence. In total, 972 patients were enrolled in the two studies, of whom 259 (26.6%) had 535 *P. vivax* recurrences (range 1-8 per patient). At 12 months, the unadjusted risk of recurrence without primaquine was 57.3%, compared to 16.5% after low-dose primaquine and 10.0% after high-dose primaquine. Across 792 initial and 468 recurrent infections genotyped, the mean number of distinct alleles per microhaplotype was 7 (range 1-25). Parasite genetic data were available for 195 (75.3%) pairs of enrolment and first recurrent infections - when unavailable treatment failure was assumed. The reinfection-adjusted risks of failure were 52.4% in patients not treated with primaquine, 12.1% in those treated with low-dose primaquine and 5.3% in those treated with high-dose primaquine - 9%, 27%, and 47% lower than the unadjusted estimates, respectively. Overall, 80% of treatment failures occurred by 4 months. Current antirelapse efficacy studies substantially overestimate primaquine failure. Bias is more pronounced for highly efficacious high-dose regimens. Our transferable probabilistic framework has the potential to transform the design and interpretation of *P. vivax* clinical trials.

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CHARACTERIZATION OF THE IN VITRO INTERACTIONS BETWEEN ANTIMALARIAL PLANTS *AZADIRACHTA INDICA* AND *ALSTONIA BOONEI* AND ARTEMISININ TO INFORM PUBLIC HEALTH POLICY.

Protus Arrey Tarkang[1], Nguele Marie Eden Olangana[2], Jeannette Nina Magoudjou-Pekam[3], Tamdem Stephanie La Blanche Guetchueng[1], Emmanuel Nnanga Nga[2], Lawrence Ayong[3]

[1]Institute of Medical Research and Medicinal Plants Studies (IMPM), Yaounde, Cameroon, [2]Faculty of Medicine and Biomedical Science, University of Yaounde 1, Yaounde, Cameroon, [3]Centre Pasteur du Cameroun, Yaounde, Cameroon

The emergence of resistance by malaria parasites to conventional antimalarial drugs has renewed the exploration of herbal medicine as alternatives. Furthermore, the increasing use of antimalarial herbal medicines such as *Azadirachta indica* and *Alstonia. boonei* in combination with conventional antimalarial drugs such as artemisinin (ART) has inspired the review of herb-drug interactions. In as much as combination therapy is recommended to ensure enhanced efficacy and probable inhibition of resistance in parasite strains, this dual use may be without recourse in the case of consequential antagonistic effects. Hence the need to characterize these interactions in view of informing public health policy. Aqueous and ethanol plant extraction of *A. indica* and *A. boonei* (AAi, Aab, EAi, EAb) were carried out by percolation. In vitro antiplasmodial activities of the plant extracts were evaluated on multidrug resistant *P. falciparum* strain, followed by cytotoxicity screening [3]. Artemisinin-plant extract interactions were analyzed using a variable (3:0 – 0:3) and equi-potency ratio combination. The 50% fractional inhibitory concentration (FIC_{50}) and combination indices (CI) were calculated from determined EC_{50} values. CI_{50} (ug/mL) values and percentage inhibitions at 100µg/mL: ART (0.01; 100), CQ (0.29; 78.33), AAb (214.8; 83.6), AAi (>500; 34.5), EAb (50.21; 100), EAi (41.11; 95.42). Calculated concentration efficacies of ART in combination with AAi at different concentrations (AAi Conc µg/mL: CE_{50} ART; fold increase in ART activity): (0: 31.57, 0), (0,25: 16.69; 1.89), (050: 19.4; 1.63), (100:14.43; 2.20) exhibiting dose dependent potentiation of ART activity. Calculated combination indices (CI) at variable and equipotency ratios and type of interaction: ART-AAb (2:1 – 0.61, 1:1 - 0.48; synergy; 1:2 – 3.52; antagonism), ART-EAb (2:1 – 1.17, 1:1 - 1.25, 1:2 – 0.80; antagonism), ART-EAi (2:1 – 0.78, 1:1 - 0.79, 1:2 – 0.75; moderate synergy), demonstrating that the combination ratio influences the type of interaction. Taken together, this evidence and further downstream analyses could likely inform policy on complementary and integrative medicine.

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ACCEPTABILITY AND FEASIBILITY OF WEIGHT-BASED TAFENOQUINE FOR THE RADICAL CURE OF *PLASMODIUM VIVAX* IN BRAZIL, ETHIOPIA, INDONESIA, AND PAPUA NEW GUINEA: A QUALITATIVE STUDY

Sarah A. Cassidy-Seyoum[1], Felipe Murta[2], Samuel Alemu Bamboro[3], Inge Sutanto[4], Erni Juwita[4], Brioni R. Moore[5], Moses Laman[6], Tamiru Shibiru Degaga[3], Maria Paula Mourao[2], Marcus Lacerda[2], Kamala Thriemer[1]

[1]Global and Tropical Health Division, Menzies School of Health Research, Darwin, Australia, [2]Fundação de Medicina Tropical Doutor Heitor Vieira Dourado, Manaus, Brazil, [3]College of Medicine & Health Sciences, Arba Minch University, Arba Minch, Ethiopia, [4]Department of Parasitology, University of Indonesia, Jakarta, Indonesia, [5]School of Diagnostic and Therapeutic Sciences, Curtin University, Bentley, Australia, [6]Papua New Guinea Institute of Medical Research, Port Moresby, Papua New Guinea

Tafenoquine is a novel treatment option for the radical cure of *Plasmodium vivax* malaria. As a single-dose treatment it offers improved adherence compared to primaquine. It is currently provided as a fixed dose of 300mg to patients above the age of 16 years, facilitating simpler treatment administration. However, the fixed dose may result in under-dosing in heavier individuals and over-dosing in lighter patients potentially affecting efficacy and safety. The TADORE - Tafenoquine Dosing Revised - clinical trial is evaluating a weight-based dosing regimen to optimize treatment outcomes. The aim of this study is to explore the acceptability and feasibility of weight-based tafenoquine, which may introduce additional complexity into routine implementation. We are conducting qualitative interviews and focus group discussions with policymakers and implementers in Brazil, Ethiopia, Indonesia, and Papua New Guinea, as well as with healthcare workers and patients in Brazil, where tafenoquine is used routinely. We explore preferences for fixed versus weight-based dosing and key operational considerations including weight-bands, pill cutting, pill burden, and packaging drawing on previous and current weight-based treatment experiences. Full results, including cross-country comparisons, will be available at the time of the conference. Findings will inform strategies to support the implementation of weight-based tafenoquine dosing and guide policy decisions on optimizing radical cure for *P. vivax* malaria.

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ENCAPSULATION APPROACHES TO IMPROVE THE PHARMACOKINETICS OF A NOVEL ANTI-MALARIAL COMPOUND, 2741-19

Aidan Biondi[1], Brooke Skinner[1], Hannah Wu[2], Tanbir Najrana[3], Fabian Fischer[4], Jonathan D. Kurtis, MD/PhD[5]

[1]Brown University, Providence, RI, United States, [2]Brown University, School of Medicine, Lincoln, RI, United States, [3]Providence, RI, United States, [4]University of Rhode Island, Kingston, RI, United States, [5]Brown University Medical School, Providence, RI, United States

Artemisinin-resistant *Plasmodium falciparum* mandates the development of novel anti-malarial drugs, yet no new chemical entities active against blood stage parasites have been approved in decades. Recently, we identified a small molecule antimalarial candidate, 2741-19, which has an IC_{50} of 50 nM for killing *P. falciparum*, a selectivity index of 500-1000, and efficacy in the humanized NSG/*P. falciparum* model. Following subcutaneous (SC) administration in rats and non-human primates, 2741-19 has distribution and elimination half-lives of 1.4 and 38 hours, respectively. At a dose

of 5 mg/kg, maintaining therapeutic trough levels (>800 nM) requires frequent dosing, which is difficult to sustain in the resource-limited endemic settings where malaria predominates. To extend the dosing interval, we have evaluated the pharmacokinetic parameters of 2741-19 in rats using several human-use acceptable delivery vehicles, including encapsulation in silica, poly(lactic-co-glycolic acid), and liposome nanoparticles, as well as a thermosensitive injectable hydrogel. Sprague-Dawley rats were injected SC with each of these formulations at 5 mg drug base/kg with serial blood sampling over 96 hours. Serum samples were analyzed by LC/MS to quantify levels of 2741-19, and data were analyzed to calculate standard pharmacokinetic parameters. The T_{max} for 5 mg/kg 2741-19 by liposome and hydrogel delivery increased to 6 hours, as compared to 1 hour for 2741-19 in PBS. Importantly, hydrogel encapsulation is expected to enable a greater than 4-fold extension of the dosing interval while maintaining therapeutic trough levels (>800 nM). We are currently evaluating these formulations at reduced dosing frequency in the NSG/*P. falciparum* mouse model.

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A COMPUTATIONAL FRAMEWORK FOR THE RATIONAL DESIGN OF CYCLIC PEPTIDES TARGETING *PLAMODIUM FALCIPARUM* MSP38

Fatoumata Gnine Fofana[1], Sergio A. Hassan[2], Oudou Diabate[3], Cheickna Cisse[4], Mahamadoun Haram Toure[1], Mahamadou Diakite[5], Seydou Doumbia[6], Mamadou Wele[1]

[1]African Center of Excellence in Bioinformatics and Data Science (ACE-Mali)/ University of Sciences, Techniques and Technologies of Bamako (USTTB), Bamako, Mali, [2]National Institute of Allergy and Infectious Diseases (NIAID)/ National Institute of Health (NIH)/ Department of Health and Human Services, Bethesda, MD, United States, [3]African Center of Excellence in Bioinformatics and Data Science (ACE-Mali)/ University of Sciences, Techniques and Technologies of Bamako (USTTB), Bamako, Mali, Bamako, Mali, [4]FST-ACE, Bamako, Mali, [5]MRTC-USTTB, Bamako, Mali, [6]University Clinical Research Center, Faculty of Medicine, BAMAKO, Mali

Merozoite surface proteins (MSPs) mediate essential protein-protein interactions required for erythrocyte invasion and represent structurally compelling yet underexplored therapeutic targets. From a curated set of 24 *Plasmodium falciparum* surface proteins, 6 MSP candidates were evaluated against criteria including functional involvement in erythrocyte invasion, inter-strain conservation, structural accessibility, and therapeutic targeting potential. MSP38 was selected for its central role in MSP1 complex assembly and direct involvement in merozoite attachment and red blood cell invasion. We present a computational workflow combining molecular dynamics (MD) simulations to explore its conformational landscape, surface topography analysis to identify binding hotspots, and an automated cyclic peptide design pipeline using RFdiffusion and ProteinMPNN. MD simulations revealed a predominantly stable structural framework. Conformational clustering identified four structurally distinct cavities with diverse physicochemical environments compatible with hydrophobic, electrostatic, and hydrogen-bonding interactions. Targeting these pockets, RFdiffusion generated geometrically complementary macrocyclic scaffolds, and ProteinMPNN optimized side chains to yield sequence-designed cyclic peptides tailored to each cavity. Analysis of protein-peptide complexes, including interaction strength and persistence and binding dynamics enabled ranking and lead identification. We further propose incorporation of non-standard amino acids at key interface positions to enhance affinity and selectivity. This study demonstrates how conformational ensemble analysis integrated with generative protein design enable rational targeting of parasite surface interfaces, establishing a broadly applicable framework for developing macrocyclic inhibitors against challenging protein-protein interfaces.

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THE MOLECULAR MECHANISM OF COOPERATIVITY OF HEME-DIHYDROARTEMISININ AND LUMEFANTRINE IN SUPPRESSING HEME DETOXIFICATION

Peter G. Vekilov

University of Houston, Houston, TX, United States

Antimalarial combination therapies are generally rationalized by pharmacokinetic complementarity, but whether paired drugs also interact directly during heme crystallization remains unclear. Here, using in situ atomic force microscopy, we show that the artemisinin-derived adduct heme-dihydroartemisinin(H-DHA) and lumefantrine (LF) produce a strongly ratio-dependent response during β-hematin growth. At H-DHA:LF molar ratios of 1:10 and 1:5, but not 1:1, the crystal surface develops mesoscopic voids together with a disordered overlayer, followed by a marked and persistent loss of normal step propagation. These features remain after prolonged exposure to drug-free growth solution, indicating irreversible surface passivation. Mechanistically, the results suggest that H-DHA lowers the threshold for LF-associated void formation, while the overlayer further impedes solute incorporation. Notably, the two drugs are antagonistic in the classical inhibition regime, yet become strongly cooperative once this altered surface state is established. Our findings show that an artemisinin-derived species and a partner drug can jointly drive hematin crystals into a persistent inactive state, and suggest that collective, nonclassical growth inhibition may contribute to the action of antimalarial drug combinations.

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INTERCALATION OF A BIOACTIVE COMPOUND INTO ZIRCONIUM PHOSPHATE NANOPARTICLES FOR POTENTIAL ANTIMALARIAL THERAPY APPLICATIONS

Ninoshka Marie Delgado Rivera[1], Adelfa E. Serrano[2], Jorge L. Colón Rivera[1]

[1]University of Puerto Rico - Rio Piedras Campus, San Juan, Puerto Rico, [2]University of Puerto Rico School of Medicine, San Juan, PR, Puerto Rico

Parasites such as the *Plasmodium* parasite of malaria are the cause of diseases that have a high mortality rate worldwide. Nanomaterials have been extensively studied as potential drug delivery agents against different diseases. The nanomaterials studied in our research laboratory are layered zirconium phosphate (ZrP) nanoparticles, which can intercalate bioactive species between their layers through direct ion exchange. ZrP nanoparticles are hexagonal, physiologically and thermally stable, target-selective, and have high drug loading capacity. The general objective of this project is to study the potential of ZrP as a nanocarrier of an antimalarial drug and to develop a compound with potentialtherapeutic application to help combat this disease. Intercalations of the antimalarial agent TCMDC was carried out at different molar ratios inside the ZrP nanoparticles (Drug:Zrp - 10:1, 5:1, 1:1. 1:5, and 1:10). The characterization techniques used were Infrared Spectroscopy (IR), X-Ray Powder Diffraction (XRPD), Energy Dispersive X-Ray Spectroscopy (EDS) by means Scanning Electron Microscopy (SEM), and Thermogravimetric Analysis (TGA). The interlaminar distance of α-ZrP is 7.6 Å. XRPD showed an increase in interlayer distance of 12.8 Å for TCMDC-loaded ZrP, suggesting that the drug was intercalated into ZrP. Further confirmation was obtained by IR, SEM-EDS, and TGA measurements. According to the characterization techniques, the best molar ratio for TCMDC:ZrP was 1:5. The first *P. berghei* blood-stage *in-vitro* assays were conducted to evaluate and compare the antiplasmodial activity of the intercalated product 1:5 TCMDC:ZrP versus the drug alone, TCMDC. In these initial assays, the intercalated product showed antiplasmodial activity similar to that of the drug alone, whereas the ZrP nanoparticles exhibited no effect on parasite growth. These results demonstrate that ZrP nanoparticles could be used as a potential drug delivery system for antimalarial drugs. In future work, drug release assays and additional repetitions of the *P. berghei* blood stages assays for 1:5 TCMDC:ZrP will be performed.

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IDENTIFICATION OF A MULTISTAGE ANTIPLASMODIAL CANDIDATE WITH A RESISTANCE-REFRACTORY PROFILE

Roberto G. Díaz-González[1], Emilee E. Colón-Lorenzo[1], Alison Roth[2], Kathy Trujillo-Nevarez[1], José G. Ortiz[1], David A. Fidock[3], Jürgen Bosch[4], Joel Vega-Rodriguez[5], Adelfa E. Serrano[1]

[1]University of Puerto Rico-School of Medicine, San Juan, Puerto Rico, [2]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [3]Columbia University Irving Medical Center, New York, NY, United States, [4]Case Western Reserve University, Cleveland, OH, United States, [5]National Institute of Allergy and Infectious Diseases, Rockville, MD, United States

The rapid emergence and spread of antimalarial drug resistance threatens global malaria control and compels the discovery of novel antimalarial therapies. During erythrocytic infection *Plasmodium* parasites are exposed to oxidative stress that is partially mitigated by the glutathione pathway. Glutathione-S-Transferase (GST), a key enzyme within this pathway, is essential for *Plasmodium* parasites and represents a potential drug target. To identify potential inhibitors of parasite GST, a structure-based virtual screening of the ChEMBL-NTD library was performed against the *Plasmodium* GST identifying 21 candidate compounds with predicted high-binding affinity. *In vitro* drug assays validated four compounds with activity against *P. berghei* asexual blood stages (EC_{50} :1-3 μM). Among these, compound A displayed potent activity against both drug-sensitive and multidrug-resistant *P. falciparum* strains (EC_{50}: 0.13-0.38 μM), indicating broad-spectrum efficacy. Beyond asexual stages, compound A inhibited *P. berghei* ookinete development *in vitro* (IC_{50} = 1 μM), showing transmission-blocking activity and multistage potency. Unexpectedly, despite being selected from a GST-targeted screening, it did not inhibit *P. berghei* GST enzymatic activity suggesting an alternate mechanism of action. Furthermore, the compound was shown to be refractory to induce drug resistance mutations in *P. falciparum*. Compound A further exhibited no hemolytic activity and a pharmacological profile comparable to chloroquine. Altogether, these findings identify compound A as a potent, multistage antiplasmodial compound with a novel mechanism of action, refractory to drug resistance, and a promising candidate for next-generation antimalarial development.

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ENTEROHEPATIC RECIRCULATION CAPTURES MULTIPHASIC PIPERAQUINE PHARMACOKINETICS IN ZAMBIAN CHILDREN WITH UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA

Sandra Massih[1], Behnoosh Bonakdar Yazdi[2], Jay Sikalima[3], Jean-Bertin Bukasa Kabuya[4], Francesca Aweeka[5], Liusheng Huang[6], Matthew M. Ippolito[7]

[1]Johns Hopkins University, Baltimore, MD, United States, [2]Baltimore, MD, United States, [3]Churches Health Association of Zambia, Ndola, Zambia, [4]Tropical Diseases Research Centre, Ndola, 8, Zambia, [5]San Francisco, CA, United States, [6]UCSF, San Francisco, CA, United States, [7]Johns Hopkins University School of Medicine, Baltimore, MD, United States

Piperaquine (PPQ), the long-acting partner drug in dihydroartemisinin-piperaquine (DP), has complex pharmacokinetics characterized by variable absorption, large distribution volume, and a long terminal half-life. We characterized PPQ pharmacokinetics (PK) in Zambian children with uncomplicated *falciparum* malaria. Children received 3 once-daily doses of DP with intensive sampling during the first 72 hours, followed by weekly levels to capture the long PPQ tail. Noncompartmental analysis informed nonlinear mixed-effects population modeling. Multicompartment models with and without lag time were evaluated, and transit compartments and enterohepatic recirculation (EHR) were explored to capture multiple peaks. Covariates included body size, age, sex, and initial parasitemia. PPQ concentration-time profiles showed marked inter-individual variability with some featuring a single peak at 2-4 hours, and others featuring a second peak at 8-12 hours. A 2-compartment model with first-order absorption, lag time (~0.5 hour), additive error, and EHR best described the data. Population estimates (95% CI) were CL/F 0.23 L/h/kg (0.22-0.25), Vd/F 2.1 L/kg (2.0-2.2), and ka 0.10 h^-1 (0.09-0.11). Interindividual variability was moderate for clearance (59% CV) and high for absorption (175% CV). A 2-compartment model with lag time and EHR best characterized PPQ PK in this pediatric population, capturing the observed multiphasic absorption. Further work will characterize exposure-response relationships between PPQ and parasite clearance across stages of the *Plasmodium* lifecycle and incidence of recurrent malaria.

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LEAD OPTIMIZATION OF MULTISTAGE ANTIMALARIAL TAMBJAMINES

Amrendra Kumar[1], Yuexin Li[2], Cecil Weale[3], Larnelle Garnie[3], Davin Hong[3], Diana Caridha[4], Michael Madejczyk[4], Chau Vuong[4], William Dennis[4], Susan Leed[4], Kristina Pannone[4], Patricia Lee[4], Tarrick Qahash[5], Douglas Shoue[5], Michael Ferdig[5], Jennifer Legac[6], Oriana Kreutzfeld[6], Philip Rosenthal[6], Kevin Reynolds[1], Alison Roth[4], David Fidock[3], Jane Kelly[2], Papireddy Kancharla[1]

[1]Portland State University, Portland, OR, United States, [2]Portland VA Medical Center, Portland, OR, United States, [3]Columbia University Irving Medical Center, New York, NY, United States, [4]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [5]University of Notre Dame, Notre Dame, IN, United States, [6]University of California San Francisco, San Francisco, CA, United States

Natural products have long served as a rich source of antimalarial agents and continue to inspire the discovery of new therapeutics. In an effort to develop new antimalarial agents with multistage activity and improved resistance liabilities, we optimized the tambjamine chemotype as a new lead series. This effort delivered compounds with potent activity against MDR *Plasmodium* strains, favorable metabolic and pharmacokinetic properties, and robust efficacy in rodent models of both blood- and liver stage malaria. In high-inoculum resistance-selection experiments, lead candidate(s) showed no recrudescence, supporting a resistance-refractory profile. Herein, we describe the structure-activity relationships and lead optimization of this series, highlighting compounds with enhanced multistage antiplasmodial potency, reduced toxicity, improved metabolic stability, and favorable pharmacokinetic properties. Overall, these findings establish the tambjamine scaffold as a promising platform for the development of next-generation antimalarial therapeutics.

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DEVELOPMENT OF A NOVEL LONG-ACTING CHEMOPROTECTIVE ANTIMALARIAL

Rozalia Dodean[1], Yuexin Li[1], Xiaowei Zhang[1], Patricia Lee[2], Diana Caridha[2], Michael Madejczyk[2], Monica Martin[2], Chau Vuong[2], William Dennis[2], Kristina Pannone[2], Michael Riscoe[1], Alison Roth[2], Papireddy Kancharla[3], Jane Kelly[1]

[1]Portland VA Medical Center, Portland, OR, United States, [2]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [3]Portland State University, Portland, OR, United States

The global spread of artemisinin-resistant *Plasmodium falciparum* and the limited options for long-term malaria prevention necessitate the development of novel chemoprotective antimalarial agents with unique mechanisms of action. A chemotherapy/chemoprotection approach to combat all stages and species of malaria parasites, with the ability to provide single dose treatment and radical cure by clearing dormant liver stage parasites to prevent relapse malaria, is of high importance and particular interest in the eradication campaign. We have developed a novel acridone antimalarial chemotype that is potent against all three main life cycle stages (liver, blood, and mosquito) of malaria parasites, with single dose cure in a rodent model as well as the potential to prevent relapsing infection and block transmission. Safety profiles, including toxicology evaluations in animals, indicated that they are well tolerated. In the current study, we investigated the potential of acridone analogs and prodrugs as long-acting injectables (LAI) for extended chemoprotection. Preliminary studies demonstrated that a single intramuscular injection of T111 and its prodrugs protected mice against blood stage challenge of malaria

infection for as long as 4 weeks. These results indicate that a single injection of acridone lead candidates can achieve sustained, protective blood concentrations. Coupled with a favorable safety profile and broad-spectrum activity across all three life stages of *Plasmodium*, T111 and its derivatives emerge as highly promising candidates for the next generation of LAI antimalarial chemoprotection.

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COMBINING REPROTRACKER® AND ZEBRAFISH ASSAYS FOR EARLY TERATOGENICITY ASSESSMENT

Beata E. Kusmider, Marina Garcia, Belen Tornesi
MMV Medicines for Malaria Venture, Meyrin, Switzerland

Malaria During Pregnancy Is a Critical Health Issue. Pregnant Women Are Uniquely Susceptible to the Parasite Due to Immunological Changes and the Tendency of *Plamodium falciparum* to Sequester in the Placenta. When a Woman Is Pregnant, Malaria Infection Poses Serious Risks, Including Severe Maternal Anemia, Premature Birth, Low Birth Weight, Miscarriage, Stillbirth, and Maternal Mortality. Despite the Recommendation for Chemoprevention With Sulfadoxine-Pyrimethamine During 2nd and 3rd Trimester, More Than One Third of the 36 Million Pregnant Women and Girls in Moderate to High Transmission Countries in the WHO African Region Were Infected With Malaria in 2024. To Reduce Disease Burden in Pregnancy, and Adverse Pregnancy and Birth Outcomes, It Is Essential That the Development of New Therapeutic and Preventive Drugs Explicitly Addresses Safety Considerations for This Population. Classical Non-Clinical Teratogenicity Assessment Consists of Embryo-Fetal Development (EFD) Studies in Two Species, Typically Conducted Relatively Late, to Support Phase II Clinical Studies. We Established a Screening Pipeline That Integrates Two Complementary in vitro Assays - the Induced Pluripotent Stem Cell-Based ReproTracker® Assay and a Zebrafish Embryo Developmental Toxicity Assay - to Flag Teratogenic Risks Early in Antimalarial Discovery. We Screened More Than 20 Antimalarial Drug Candidates and Compared the Results to Subsequent Animal EFD Study Outcomes. We Showed That, When Combined, This Battery Was Highly Predictive of the Results of Animal EFD Studies. In Particular, It Correctly Flagged Most Compounds Later Confirmed as Teratogenic in Animals, Enabling Data-Driven Decisions to Front-Load Pivotal EFD Studies to De-Risk Development. Our Results Support Integration of These Assays in Malaria Drug Discovery, and Potentially Other Infectious Diseases, to Prioritize the Development of Much-Needed Therapeutics Which Could Benefit All Populations.

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POTENT ANTIPLASMODIAL ACTIVITY OF EXTRACTS FROM AN ENDEMIC PUERTO RICAN PLANT

Freddie John Ortiz Colón[1], Reydiliz Nieves Acevedo[2], Emilee Colón Lorenzo[1], Roberto Díaz-González[1], Jose Ortiz[1], Alison Roth[3], Claudia A. Ospina[2], Adelfa E. Serrano[1]
[1]University of Puerto Rico, Medical Sciences Campus, San Juan, PR, United States, [2]Inter American University of Puerto Rico, Bayamon, PR, United States, [3]Walter Reed Army Institute of Research, Silver Spring, MD, United States

Malaria remains a major public health challenge, and the emergence of drug-resistant *Plasmodium falciparum* strains threatens the effectiveness of current therapies. Natural products represent a valuable source of new agents that can inhibit malaria parasites. This study aimed to evaluate the antiplasmodial activity of endemic Puerto Rican plant extracts against two malaria parasites: the rodent parasite *Plasmodium berghei* and the human parasite *Plasmodium falciparum*. Parasite growth inhibition during the erythrocytic stage was measured using an *in vitro* luminescence assay in *P. berghei* alongside chloroquine as a positive control. The aqueous extracts showed inhibitory activity with IC_{50} values ranging from 0.022 to 0.734 µg/mL, while selected purified compounds (COM-01, COM-02, and COM-03) demonstrated potent nanomolar activity at 15.04, 18.08, and 10.05 nM, respectively. To determine transmission-blocking potential, inhibition of ookinete formation was assessed, with aqueous extracts showing IC_{50} values ranging from 0.00934 to 0.01365 µg/mL. Antiplasmodial activity against *P. falciparum* was also evaluated using an in vitro SYBR Green fluorescence-based assay. No significant hemolysis was observed at concentrations up to 1000 µg/mL. Cytotoxicity in HepG2 cells was also assessed, indicating a favorable safety profile. These findings highlight the potential of purified compounds from the plant as promising antimalarial leads for further evaluation.

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ENQUÊTE SUR UNE ÉPIDÉMIE DE PALUDISME DANS LE DISTRICT SANITAIRE DE PÔ, RÉGION CENTRE SUD, BURKINA FASO, 2024

Alphonse Traore[1], Ousseni Ouédraogo[1], Charles KI-Zerbo[1], Berenger Kaboré[2], Denis yelbeogo[2], Hamadou Seogo[2], Sidzabda Kompaore[1]
[1]Secretariat Permanent pour l'élimination du Paludisme, Arlington, VA, United States, [2]African Field Epidemiology Network (AFENET), Arlington, VA, United States

Le paludisme demeure un problème majeur de santé publique au Burkina Faso, où la transmission est saisonnière et atteint son pic pendant la saison des pluies. La région Centre-Sud, comme les autres régions du pays, connaît des recrudescences de paludisme récurrentes durant la saison de forte transmission. Au cours de la semaine épidémiologique 39 de 2024, le district sanitaire de Pô a signalé 3 767 cas de paludisme non compliqué et deux décès, l'incidence dépassant le seuil d'alerte épidémique. Une enquête a été menée afin de confirmer l'épidémie et d'appuyer la réponse du district. Une étude descriptive transversale a été menée du 28 octobre au 3 novembre 2024 à Tiébélé, Guiaro et Dakola, dans le district sanitaire de Pô. La population étudiée comprenait des membres de la communauté, des agents de santé et des bénévoles de santé communautaires du centre médical de Tiébélé et des centres de soins primaires de Guiaro et Dakola. Les données ont été recueillies par analyse documentaire et entretiens individuels à l'aide de formulaires standardisés. Au total, 731 cas de paludisme ont été étudiés. Parmi eux, 98 % présentaient de la fièvre ou des antécédents de fièvre et ont été confirmés par un test de diagnostic rapide positif. Les cas provenaient principalement des villages de Corabié et Tiyalo, dans le secteur sanitaire de Tiébélé, et de Guiaro. L'incidence du paludisme non compliqué était de 40 pour 1 000 habitants à Tiébélé, de 29 pour 1 000 à Guiaro et de 15 pour 1 000 à Dakola. L'âge moyen des patients était de 13,5 ans (écart-type : 15,47), avec un sex-ratio de 1,07. La couverture vaccinale contre le paludisme (RTS,S) était de 47,2 %, celle de la chimioprévention saisonnière du paludisme (CSP) de 64,0 % et 90,5 % des femmes enceintes avaient reçu la troisième dose du traitement préventif intermittent (TPIg3). Cette enquête a confirmé une épidémie de paludisme à Tiébélé, Guiaro et Dakola, dans le district sanitaire de Pô. Le renforcement de la surveillance et de la collaboration transfrontalière avec le Ghana voisin est essentiel pour prévenir de futures épidémies.

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NINETEENTH-CENTURY BRITISH WEST INDIES SOLDIERS WERE NOT INTRINSICALLY RESISTANT TO DISEASE MORTALITY IN WEST AFRICA

G. Dennis Shanks
Australian Defence Force Malaria and Infectious Disease Institute (ADFMIDI), Enoggera, Australia

Expatriate soldiers often died at high rates due to infectious diseases when deployed into the tropics during the 19th century. A British regiment of Black soldiers from the West Indies was specifically raised to provide soldiers less likely to die from malaria and yellow fever than their European counterparts. Black soldiers were initially recruited from recovered slaves born in West Africa but this changed by mid-century into free men born in the West Indies. All-cause (ranged from 1 to 6% per year) and disease specific annual mortality rates were collected from British West Indies soldiers while stationed in West Indies (Jamaica, Windward Islands) and West Africa

(Sierra Leone, Ghana). British West Indies Regiment soldiers died at lower rates initially in West Africa where they had been born. However, during the latter half of the 19th century, soldiers originating in the West Indies died at higher rates when stationed in West Africa than the West Indies due to diseases including malaria, tuberculosis, diarrheal and respiratory diseases. This discrepancy equalized by the early 20th century suggesting that resistance to disease mortality depended on early life experience with infections rather than genetic factors inherited from earlier generations from West Africa.

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HUMAN MOBILITY SHAPES *PLASMODIUM VIVAX* POPULATION STRUCTURE IN THE NORTE DE SANTANDER MIGRATION CORRIDOR, COLOMBIA

Hugo Oswaldo Valdivia[1], Fredy E. Villena[2], Carolina Cardenas[3], Claudia A. Parada-Higuera[3], Maria U. Gonzales-Pabon[3], Fabian N. Montañez[4], Lucy A. Jaimes[5], Lady Y. Gutiérrez-Silva[6], Juan Francisco Sanchez[7], Bret N. Peterson[1], Berlin L. Londono[8]

[1]U.S Naval Medical Research Unit SOUTH, Callao, Peru, [2]Lima, Peru, [3]Universidad de Pamplona, Pamplona, Colombia, [4]E.S.E Hospital Regional Norte-Tibu, Tibu, Colombia, [5]E.S.E. Hospital Jorge Cristo Sahium, Villa del Rosario, Colombia, [6]E.S.E Hospital Emiro Quintero Cañizares, Ocaña, Colombia, [7]U.S. Naval Medical Research Unit SOUTH (NAMRU SOUTH), Callao, Peru, [8]Tulane University, New Orleans, LA, United States

Colombia, along with Brazil and Venezuela, is among the countries reporting 75% of all malaria cases in the Americas, with *Plasmodium vivax* accounting for over 60% of all malaria cases in the country. The Department of Norte de Santander, Colombia ranks third in migrant population from the Bolivarian Republic of Venezuela at approximately 328,000 as of 2024. Although this region is subject to anthropogenic factors including travel and forced migration, studies examining their impact on malaria transmission dynamics remain scarce.We conducted a cross-sectional passive surveillance study in several health facilities in the Norte de Santander Department between 2019 and 2022. We collected demographic, clinical, and epidemiological data as well as blood samples from subjects with suspected malaria. Clinical specimens were used for targeted high-throughput sequencing to characterize genetic diversity, population structure, and putative drug resistance markers (Pvmdr1, Pvdhfr, PvDHPS). Our study revealed a low *P. vivax* genetic diversity (n=177, π=0.175) without evidence of temporal or geographic population structure. In this regard, our results point to high gene flow and genetic admixture consistent with high migration rates in this region. Notably, all sequenced specimens were wild-type for putative drug resistance markers (Pvmdr1, Pvdhfr); however, mutations in the PvDHPS gene were highly prevalent (91.4%).Our findings suggest that circulating *P. vivax* in Norte de Santander behaves as a highly interconnected population, likely driven by intense human mobility across the border. Our results highlight the complexity of *P. vivax* transmission dynamics along this major migratory corridor, posing significant challenges to regional malaria control and elimination efforts.

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PREVALENCE, INCIDENCE, AND RISK FACTORS FOR ASYMPTOMATIC MALARIA IN A LOW-ENDEMIC AREA IN THE PERUVIAN AMAZON REGION, 2019 - 2022

Juan F. Sanchez[1], Hugo O. Valdivia[1], Christie A. Joya[1], Danielle L. Pannebaker[1], Maria J. Saenz[2], Greys E. Braga[3], Andres G. Lescano[4]

[1]U.S. Naval Medical Research Unit SOUTH (NAMRU SOUTH), Callao, Peru, [2]Culmen International, LLC, Alexandria, VA, United States, [3]U.S. Naval Medical Research Unit SOUTH (NAMRU SOUTH), Iquitos, Peru, [4]Universidad Peruana Cayetano Heredia, San Martín de Porres, Peru

Malaria remains an endemic disease worldwide. Despite a decrease in the Americas, malaria still causes around 600,000 annual cases. Microscopy is the standard diagnostic in most endemic regions, and most cases are identified by passive surveillance. However, studies show a predominance of asymptomatic and submicroscopic malaria, highlighting the need for active surveillance and more sensitive diagnostics. Our objective was to assess the prevalence, incidence and risk factors of asymptomatic malaria in a low-endemic region of Loreto, Peru. A prospective cohort study with four visits was conducted in Padrecocha, Loreto, from 2019 - 2022. Enrollment used active surveillance. Data and blood samples were collected for malaria diagnosis by microscopy and molecular methods. We estimated the prevalence and incidence of asymptomatic malaria at baseline and follow-ups. Two multivariate models were performed to identify risk factors for malaria positivity: a conventional logistic regression with a nested model approach and a matched case-control study. Both models used participants who tested positive for malaria by microscopy and/or molecular methods at any point. We enrolled 545 participants: 37 specimens from 33 participants were positive for malaria via loop-mediated isothermal amplification (LAMP). All positive cases were asymptomatic, reporting no symptoms two weeks before the visits. The overall prevalence of asymptomatic malaria was 2.9% with incidence rates varying from 1.7% to 4.1% during follow-ups. The nested model associated four factors with asymptomatic malaria: being female (OR: 2.89; 95% CI: 1.25 - 6.68, p=0.013), a previous malaria episode in the last 12 months (OR: 3.36; 95% CI: 1.38 - 8.22, p=0.008), proximity to a water source at sunset or dawn (OR: 2.96; 95%CI: 1.40 - 6.25, p=0.004), and spraying the house with insecticide (OR: 5.71; 95% CI: 2.02 - 16.17, p=0.001). The low malaria prevalence in Padrecocha and its predominantly submicroscopic, asymptomatic presentation highlight the need for more sensitive, deployable diagnostic like LAMP, in addition to active case detection, especially during the elimination phase.

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SEASONAL DYNAMICS OF ASYMPTOMATIC *PLASMODIUM VIVAX* INFECTIONS AND MOSQUITO EXPOSURE MARKERS IN AN AMAZONIAN COMMUNITY IN PERU

Macarena Vittet-Mondoñedo[1], Lorena Tapia[1], Katherine Jessica Torres Fajardo[2], Pamela Rodriguez[2], Berlin L. Londono[3], Juan Francisco Sanchez[1], Joseph M. Vinetz[4], Bret N. Peterson[1], Dionicia Gamboa[2], Hugo Oswaldo Valdivia[1]

[1]U.S. Naval Medical Research Unit SOUTH, Callao, Peru, [2]Universidad Peruana Cayetano Heredia, Lima, Peru, [3]Tulane University, New Orleans, LA, United States, [4]Yale University, New Haven, CT, United States

Residual *Plasmodium vivax* transmission is a major challenge for malaria elimination in the Peruvian Amazon, especially in isolated communities where access to diagnosis and treatment is limited. In this setting, asymptomatic infections may persist undetected, contributing to hidden parasite reservoirs and sustaining transmission, affecting both civilian and military populations. Understanding seasonal infection patterns and mosquito exposure may reveal transmission dynamics in remote Amazonian communities. We conducted a cross-sectional survey with paired seasonal sampling in Santa Emilia, a rural Amazonian community in Peru's the Loreto region. From 204 enrolled subjects, 179 had samples available for molecular and serological analyses. *Plasmodium* infections were detected using SYBR Green real-time PCR targeting the COX-III gene, with species confirmation using a TaqMan qPCR assay targeting the 18S rRNA gene. To evaluate human-vector contact, we measured IgG antibody responses to *Anopheles* (*Anopheles* subgenus marker gSG6-P1 and *Nyssorynchus* subgenus AnDar-Apy2) using an *Aedes aegypti* Nterm-34 kDa salivary peptide as a control. Seasonal differences were evaluated using the Wilcoxon signed-rank test on 145 individuals with paired samples from the rainy (April–May) and dry (October) seasons. *P. vivax* infections were found in nine individuals during the rainy season (6.2%) and six during the dry season (4.1%). Fourteen of these infections were asymptomatic (93.3%). Antibody responses to mosquito exposure markers differ significantly between the rainy and dry seasons ($p < 0.05$). The presence of asymptomatic *P. vivax* infections highlights a hidden parasite reservoir that may sustain transmission in Amazonian communities. Combining Molecular diagnostics with serological markers of mosquito exposure may improve detection of asymptomatic infections and help characterize seasonal transmission dynamics in the Peruvian Amazon.

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SEROLOGICAL MARKERS OF RECENT EXPOSURE IDENTIFY POTENTIAL *PLAMODIUM VIVAX* MALARIA RESERVOIRS IN A LOW-TRANSMISSION AREA IN THE PERUVIAN AMAZON

Renzo Lopez-Tito[1], Macarena Vittet-Mondoñedo[2], Dionne C Argyropoulos[3], Joseph M. Vinetz[4], Hugo O. Valdivia[5], Rhea J Longley[3], Ivo Mueller[3], Dionicia Gamboa[6], Katherine Torres[6]

[1]Laboratorio ICEMR-Enfermedades Emergentes, Laboratorios de Investigación y Desarrollo, Facultad de Ciencias e Ingeniería, Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Culmen International, LLC, Alexandria, VA, United States, [3]Infection and Global Health Division, The Walter and Eliza Hall Institute of Medical Research, Parkville, Australia, [4]Section of Infectious Diseases, Department of Internal Medicine, Yale School of Medicine, New Haven, CT, United States, [5]U.S Naval Medical Research Unit SOUTH, Lima, Peru, [6]Departamento de Ciencias Celulares y Moleculares, Facultad de Ciencias e Ingeniería, Universidad Peruana Cayetano Heredia, Lima, Peru

Plsmodium vivax malaria represents a high disease burden globally. In the Peruvian Amazon 28 051 cases were reported in 2024, accounting 84.5% of total malaria cases. In this region, *P. vivax* malaria is characterized by submicroscopic and asymptomatic infections and additionally, the existence of latent liver stages (hypnozoites) constitutes the disease reservoir and poses a challenge for malaria elimination. Due to the lack of diagnostic tools for hypnozoites, serological markers of *P. vivax* recent exposure can indirectly detect individuals at risk of relapse therefore identifying the potential hypnozoite reservoir. This work aimed to determine the seroprevalence of recent exposure to *P. vivax* malaria in a remote community in the Peruvian Amazon and identify epidemiological factors associated with seropositivity. We measured IgG antibody responses against eight antigens (PvEBP-II, Pv-fam-a, PvMSP5, PvMSP1-19, PvMSP8, PvRBP2b, PvPTEX150 and PvCSS) and seropositivity was estimated using a previously trained random forest algorithm based on individual IgG responses. The overall cumulative seroprevalence of recent exposure was 66.7% [95% CI: 61.2% - 71.7%] (214/321), while prevalence by qPCR was 4.3% [95% CI: 2.5% - 7.3%] (14/321) and by microscopy was 1.6% [95% CI: 0.5% - 3.8%] (5/321). Multivariate analysis using a logistic regression model identified that age (aOR: 1.06 [1.02 - 1.11], p = 0.001), being a farmer (aOR: 6.24 [1.53 - 32.42], p = 0.013), and having a past malaria infection (aOR: 2.67 [1.46 - 4.95], p = 0.001) were associated with a higher likelihood of seropositivity, while bed net use (aOR: 0.76 [0.65 - 0.88] , p < 0.001) was associated with a lower likelihood, seasonality was not associated with seropositivity (p = 0.51). Furthermore, spatial correlation analysis using the Getis-Ord Gi* index for hotspot analysis did not identify significant clusters, indicating diffuse and homogeneous transmission within the community. These results suggest a high proportion of reservoirs in a remote “low transmission” area of the Peruvian Amazon, contributing to sustained parasite transmission.

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FACILITY-BASED SEROLOGICAL SURVEILLANCE REVEALS SPATIOTEMPORAL DYNAMICS OF HIDDEN *PLASMODIUM VIVAX* TRANSMISSION IN SULTAN KUDARAT, PHILIPPINES

Rihana A. Aydin[1], Jhobert Bernal[2], Maria Lourdes Macalinao[3], Katherine Cosca[3], Beaulah L. Boncayao[3], Ivo Mueller[2], Fe Esperanza Caridad Espino[3], Jennifer Sampang Luchavez[3], Rhea Longley[2], Gillian H. Stresman[1]

[1]University of South Florida, Tampa, FL, United States, [2]The Walter and Eliza Hall Institute of Medical Research, Parkville, Australia, [3]Research Institute for Tropical Medicine, Muntinlupa Alabang, Philippines

Plasmodium vivax elimination in the Asia-Pacific requires identifying cryptic transmission driven by dormant hypnozoites that evade conventional surveillance. Sultan Kudarat, Philippines experienced consecutive *P. vivax* outbreaks in 2020-2021 despite declining case detection, indicating undetected transmission persists. We present results from an 18-month facility-based serological surveillance study mapping the spatial and temporal distribution of residual *P. vivax* exposure. The SMaRT (Serological Markers for Residual Malaria Transmission) study enrolled 5,085 participants (4,106 care-seekers, 979 companions) across 15 barangays during rolling surveys every two months (March 2024-September 2025). Participants were enrolled regardless of consultation reason. Dried blood spots were assayed using a validated 8-marker *P. vivax* IgG Luminex panel detecting recent exposure (prior 9 months, 81% sensitivity/specificity), and households remotely geolocated via satellite imagery. Despite active case-finding, all 5,083 rapid diagnostic tests were negative, demonstrating conventional surveillance limitations. Serological analysis of all 5,085 samples reveals hidden *P. vivax* transmission: overall seroprevalence was 7.1% (preliminary analysis of 3,444 samples: 246 seropositive), with marked spatial and temporal heterogeneity across barangays. Space-time scan statistics identified significant transmission clusters, with some foci persisting across multiple survey rounds while others emerged or resolved. Mixed-effects logistic regression controlling for spatial autocorrelation identified key risk factors for recent exposure, including age, sex, travel history, and bed net non-use. This facility-based strategy uncovered hidden *P. vivax* transmission that routine surveillance cannot capture. These findings demonstrate a practical, scalable approach to identifying residual transmission foci and high-risk populations requiring targeted radical cure strategies, directly informing elimination efforts across the Asia-Pacific region.

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CLINICAL-EPIDEMIOLOGICAL PROFILE AND OBSTETRIC-NEONATAL OUTCOMES IN PREGNANT WOMEN WITH *PLAMODIUM VIVAX* MALARIA IN NORTHWEST COLOMBIA

Carlos J. Castro-Cavadía[1], Jaime Carmona-Fonseca[2], Maria Fernanda Yasnot Acosta[1]

[1]Universidad de Córdoba, Monteria, Colombia, [2]Universidad de Antioquia, Medellín, Colombia

Plamodium vivax infection during pregnancy poses significant maternal and fetal risks, particularly in endemic regions where millions of pregnancies are exposed annually; however, limited data exist regarding the clinical-epidemiological profile and obstetric-neonatal outcomes associated with gestational vivax malaria in Colombia despite sustained transmission in northwestern areas of the country. A study was conducted between 2016 and 2019 in the municipalities of Puerto Libertador and Tierralta, located in northwestern Colombia, analyzing a total of 165 samples including specimens from 79 pregnant women, 43 placentas, and 43 newborns to evaluate clinical manifestations, laboratory parameters, and obstetric-neonatal outcomes in pregnant women with confirmed *P. vivax* infection. Among pregnant women with vivax malaria, the classic symptomatic triad was present in 76% of cases, with headache (100%), chills (90%), and fever (82%) being the most frequently reported symptoms, while statistically significant differences were observed in laboratory parameters when compared to non-infected pregnant women except for indirect bilirubin and aspartate aminotransferase levels. No adverse obstetric outcomes including abortions, maternal deaths, or stillbirths were documented, although six newborns presented with low birth weight with no other major neonatal complications recorded. This study characterizes the clinical-epidemiological profile of *P. vivax* infection in pregnant women from northwestern Colombia and demonstrates that despite a substantial symptomatic burden, no severe adverse obstetric outcomes were observed, underscoring the importance of early diagnosis and continuous follow-up to mitigate the risks associated with gestational malaria in endemic settings.

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MALARIA DRIVES ANAEMIA SEVERITY IN PREGNANCY: INSIGHTS FROM COMMUNITIES ALONG THE TONO IRRIGATION SCHEME IN NORTHERN GHANA

Sylvester Donne Dassah[1], Naja Kwayaja[1], Dodzi Kwaku Jnr Senoo[2], Francis Yennube Duut[1], Millicent Captain-Esoah[2], Thomas A. Anyorigiya[3], Lucas Naam-Kayagre Amenga-Etego[4], James D. Abugri[1], Godfred Agongo[1]

[1]Department of Biochemistry and Forensic Sciences, School of Chemical and Biochemical Sciences, University of Technology and Applied Sciences, Navrongo, Ghana, [2]Department of Applied Biology, School of Environment and Life Sciences, University of Technology and Applied Sciences, Navrongo, Ghana, [3]Department of Microbiology and Immunology, School of Medical Sciences, University of Technology and Applied Sciences, Navrongo, Ghana, [4]West African Centre for Cell Biology of Infectious Pathogens (WACCBIP), Department of Biochemistry, Cell and Molecular Biology, College of Basic and Applied Sciences, University of Ghana, Legon, Accra, Ghana

Anaemia in pregnancy remains prevalent in sub-Saharan Africa. Although multiple parasitic infections contribute, the role of malaria in determining anaemia severity is not well explained in northern Ghana. A hospital-based cross-sectional study was conducted among 307 pregnant women in communities along the Tono irrigation scheme. Haemoglobin concentration was measured using a portable haemoglobin meter. Malaria was diagnosed by microscopy, while urine and stool samples were examined for Schistosoma spp. and soil-transmitted helminths (STHs) using urine centrifugation and Kato Katz techniques, respectively. Logistic regression assessed associations with anaemia and its severity. Mean haemoglobin was 9.9 ± 1.43 g/dL, with anaemia prevalence of 86.6%. Malaria prevalence was 9.45%, compared with 10.42% for STHs and 4.23% for schistosomiasis. All women with malaria or co-infections were anaemic. Malaria was not significantly associated with anaemia occurrence but was strongly associated with increased severity (AOR = 7.9; $p < 0.01$). Helminth infections showed no significant association. Rural residence increased the odds of anaemia. Malaria is a key driver of anaemia severity in pregnancy despite moderate prevalence. Strengthening malaria prevention within antenatal care is critical to reducing severe maternal anaemia in endemic settings.

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PCR-BASED MOLECULAR DETECTION AND GENETIC DIVERSITY OF HUMAN *PLASMODIUM* SPECIES IN FIVE MOSQUITO-ENDEMIC COMMUNITIES IN THE EASTERN REGION OF GHANA

Kofi Agyapong Addo[1], Austine Tweneboah[2], Thomas Kwame Addison[3], Mahamat Alhadj Moussa Ibrahim[4], Soerge Kelm[5], Kingsley Badu[6]

[1]University of Skills Trianing and Entrepreneurial Development, Kumasi, Ghana, [2]University of Cologne, Cologne, Germany, [3]University of Cape Coast, Cape Coast, Ghana, [4]University of N'Djamena, N'Djamena, Chad, [5]University of Bremen, Bremen, Germany, [6]Kumasi, AH, Ghana

Malaria remains a significant public health challenge among populations in rural communities, despite considerable financial investments and widespread interventions implemented at both local and global levels to reduce its transmission. This study sought to determine the molecular prevalence and genetic diversity of human *Plasmodium* spp in five mosquito-endemic communities in the Eastern region of Ghana.In a cross-sectional community-based study involving five (5) communities (Nkatenkwan, Ayekokooso, Kwahyia, Santramor 1 and Zorh) in Ghana, two (2) mL venous blood samples were collected from 240 participants who met the inclusion criteria and signed a consent or assent form. The techniques used include: questionnaire interviews, nucleic acid assays for screening participants (DNA extraction, DNA amplification of *Plasmodium* ssRNA genes using species-specific primers), gel electrophoresis and anthropometric data analysis from questionnaire data obtained from study participants.An observed prevalence of 49.6% for human *Plasmodium* DNA *was* recorded. The *Plasmodium* DNA identified were *P. falciparum (71.4%)*, *P. malariae (7.6%) and P. ovale (0.8%)*. Some of the participants also had more than one (1) *Plasmodia* DNA, with *P. falciparum* and *P. malariae* DNA being the most prevalent mixed infections seen. Mixed infections involving *P. ovale* were the least recorded at 1.7%. *Plasmodium* DNA was most frequently observed in the 11-20 Age category, at 69.2%. Zorh recorded the highest *Plasmodium* DNA of 56.2%. There was no association between having a fever in the past 2 days and malaria infection. Not sleeping under an insecticide-treated net was associated with an increased odds of malaria infection.The study area recorded a high prevalence of malaria among participants, highlighting the urgent need to strengthen surveillance systems and intensify control strategies to reduce the burden of the disease on human health.

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INCREASED *PLASMODIUM FALCIPARUM* PREVALENCE IN THE DEMOCRATIC REPUBLIC OF THE CONGO: EVIDENCE FROM A NATIONAL MOLECULAR ANALYSIS OF THE 2023-2024 DEMOGRAPHIC AND HEALTH SURVEY

Fabien Zimbombe Vulu[1], Jenna Zuromski[2], Christopher Michael Hennelly[3], Benita Bwebwe[4], Grace Mwakole[4], Tania Lomingo[4], Anicet Kwabayina[4], Gedeon Bongo[4], Linda James[5], Jonathan J. Juliano[6], Joris Losimba Likwela[7], Baudouin Baangi Matela[8], Melchior Mwandagalirwa Kashamuka[9], Eric Mafuta[1], Antoinette Tshefu Kitoto[10], Mumba Dieudonne[1], Jonathan Parr[11]

[1]University of Kinshasa, Kinshasa, Republic of the Congo, [2]Brown University, Providence, RI, United States, [3]Chapel Hill, NC, United States, [4]National Institute of Biomedical Research, Kinshasa, Republic of the Congo, [5]University of North Carolina, Chapel Hill, NC, United States, [6]Univ of North Carolina, Chapel Hill, NC, United States, [7]National Malaria Control Programme of DRC, KInshasa, Republic of the Congo, [8]General Secretary of Public Health, DRC, Durham, NC, United States, [9]Kinshasa, Republic of the Congo, [10]Kinshasa School of Public Health, Kinshasa, United States, [11]University of North Carolina at Chapel Hill, Chapel Hill, NC, United States

Despite decades of malaria control efforts, the burden of *Plasmodium falciparum* remains high in the Democratic Republic of the Congo (DRC). An estimated 12% of the world's malaria cases occur in the DRC, yet nationally representative data on parasite prevalence and antimalarial resistance are limited. The 2023-24 DRC Demographics and Health Study (DHS) found 33% malaria prevalence in children 6-11 months by rapid detection testing. The purpose of our molecular study is to estimate prevalence among both children and adults, as well as to detect genetic markers of antimalarial drug resistance in a representative subsample of 17,705 dried blood spots collected from the 2023–2024 Demographic and Health Survey (DHS), including children aged 6–11 months, women aged 15–49 years, and men aged 15–54 years. DNA was extracted from dried blood spots using Chelex-Tween, and *P. falciparum* infection was detected by quantitative PCR (qPCR) targeting the Pf lactate dehydrogenase gene. Positive samples were assessed for drug-resistance markers using the Pf-SMARRT amplicon deep sequencing panel. Associations with covariates such as age and sex were evaluated using chi-square tests. To date, qPCR has been completed on 14,341 samples. Overall weighted *P. falciparum* prevalence by qPCR was 43.1% (95% CI: 40.5–45.7), increased from 30.3% in the 2013 DHS. The province-level prevalence ranged from 13.9% in Kinshasa to 68.2% in Tanganyika. Positivity differed significantly by sex (45.7%, 95% CI: 42.8–48.7 in males vs 40.6%, 95% CI: 37.7–43.5 in females; $\chi^2 = 37.9$, $p < 0.001$). Prevalence also differed by age group (<5 vs ≥5 years; $\chi^2 \approx 16.0$, $p < 0.001$), with higher positivity among individuals aged ≥5 years. Drug-resistance marker sequencing is ongoing. Our preliminary results show that *P. falciparum* infection prevalence is increasing in the DRC despite malaria control efforts, affecting nearly half of the population. Infection and drug-resistance marker prevalence maps will be used to inform control efforts. Sustained investment and novel strategies are required to combat malaria's heavy toll in the DRC.

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MALARIA CONTROL AND THE UNEXPECTED SPREAD OF DIAGNOSTIC-RESISTANT *P. FALCIPARUM* IN PERU

Isabela Gerdes Gyuricza[1], Abebe Alemu Fola[2], Alfred Simkin[3], Kyaw L. Thwai[1], Ablah M. Siddiq[1], Jonathan J. Juliano[4], Jeffrey A. Bailey[5], Parul Johri[1], Cobi M. Henry[1], Luis Cabrera-Sosa[6], Gerardo Porras-Laymito[6], Qin Cheng[7], Oliver John Watson[8], Dionicia Gamboa[9], Hugo Oswaldo Valdivia[10], Jonathan Parr[1]

[1]University of North Carolina at Chapel Hill, Chapel Hill, NC, United States, [2]TNU, Providence, RI, United States, [3]Brown University, Raleigh, NC, United States, [4]Univ of North Carolina, Chapel Hill, NC, United States, [5]Brown University, Providence, RI, United States, [6]Universidad Peruana Cayetano Heredia, Lima, Peru, [7]Australian Defence Force Malaria and Infectious Disease Institute, Brisbane, Australia, [8]Imperial College London, London, United Kingdom, [9]Universidad Peruana Cayetano Heredia, San Martín de Porres, Peru, [10]U.S Naval Medical Research Unit SOUTH, Lince, Peru

Plasmodium falciparum parasites with deletions in the histidine-rich protein 2 and 3 (*hrp2* and *hrp3*) genes evade detection by common rapid diagnostic tests (RDTs), posing a threat to malaria control. The evolutionary forces driving the spread of these diagnostic-resistant strains remain unclear. We sequenced 1,215 *P. falciparum* samples collected between 2003 and 2018 in Loreto, Peru. This region experienced a major decline in malaria transmission following the Project for Malaria Control in Andean Border Areas (PAMAFRO) and now harbors a high proportion of *hrp2/3*-deleted parasites despite limited RDT use. Using a genome-wide molecular inversion probe sequencing panel, we observed a reduction in genetic diversity over time and fixation of *hrp2–/hrp3–* (double-deleted) parasites. Identity-by-descent analysis revealed expansion of a dominant double-deleted lineage, consistent with clonal replacement following intensive malaria control. To investigate the evolutionary forces underlying the fixation of these deletions, we simulated allele frequencies under different selection coefficients. We found that fixation of *hrp2-/3-* due solely to genetic drift (selection coefficient $s = 0$) is unlikely; a selection coefficient of $s \geq 0.03$ was required for fixation to occur consistently. However, our simulations also indicate that a genetic bottleneck caused by PAMAFRO increased the likelihood of fixation through drift by 4.5- to 17-fold depending on the population. These findings suggest that hrp2-/3- fixation was likely driven by a combination of demographic changes resulting from PAMAFRO and selective advantage unrelated to RDT use. Our results demonstrate how intensive malaria control efforts can reshape parasite populations and underscore the value of expanded genomic surveillance as countries move toward malaria elimination.

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GENOMIC MALARIA SURVEILLANCE TO UNRAVEL POPULATION STRUCTURE AND PARASITE CONNECTIVITY OF *PLASMODIUM VIVAX* POPULATIONS IN LATIN AMERICA

Luis Cabrera-Sosa[1], Johanna H. Kattenberg[2], Mathijs Mutsaers[2], Winnie A. Ladeia[3], Milagros Saavedra-Samillán[4], Jhon Zumaeta[5], Carlos J. Castro-Cavadía[6], Maria Fernanda Yasnot[6], M. Andreina Pacheco[7], Ananias A. Escalante[7], Fabián E. Saénz[8], Gustavo Fontecha[9], Alberto Tobón-Castaño[10], Hugo O. Valdivia[11], Stella M. Chenet[4], Emelie Javelle[12], Marcelo U. Ferreira[3], Christopher Delgado-Ratto[13], Dionicia Gamboa[1], Anna Rosanas-Urgell[2]

[1]Instituto de Medicina Tropical "Alexander von Humboldt", Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Department of Biomedical Sciences, Institute of Tropical Medicine Antwerp, Antwerp, Belgium, [3]Department of Parasitology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil, [4]Instituto de Investigación de Enfermedades Tropicales, Universidad Nacional Toribio Rodríguez de Mendoza de Amazonas, Amazonas, Peru, [5]Laboratorio de Malaria: Parásitos y Vectores, Laboratorios de Investigación y Desarrollo, Facultad de Ciencias e Ingeniería, Universidad Peruana Cayetano Heredia, Lima, Peru, [6]Grupo de Investigaciones Microbiológicas y Biomédicas de Córdoba (GIMBIC), Universidad de Córdoba, Córdoba, Colombia, [7]Biology Department, Institute of Genomics and Evolutionary Medicine, Temple University, Philadelphia, PA, United States, [8]Centro de Investigación para la Salud en América Latina, Pontificia Universidad Católica del Ecuador, Quito, Ecuador, [9]Instituto de Investigaciones en Microbiología, Universidad Nacional Autónoma de Honduras, Tegucigalpa, Honduras, [10]Grupo Malaria, Facultad de Medicina, Universidad de Antioquia, Medellín, Colombia, [11]U.S Naval Medical Research Unit SOUTH, Lima, Peru, [12]Unité Parasitologie et Entomologie, Département Risques Vectoriels, Institut de Recherche Biomédicale des Armées, CNR sud du paludisme, Aix Marseille Univ, SSA, APHM, RITMES, Marseille, France, [13]Malaria Research Group (MaRch), Global Health Institute, University of Antwerp, Antwerp, Belgium

Plasmodium vivax is the predominant malaria species in Latin America (LATAM), accounting for 63% of cases in 2024. Genomic surveillance tools are increasingly important for malaria control and elimination programs to characterize transmission dynamics, assess connectivity between countries, and monitor and molecular resistance markers. Here, we applied the Pv AmpliSeq v2 Peru assay, an amplicon-based sequencing tool for molecular surveillance, to *P. vivax* populations across LATAM. We genotyped samples from Peru (n=343), Brazil (100), Ecuador (35), Colombia (19), Venezuela (49), Honduras (6), and French Guiana (24) using this assay. Genomic data were analyzed to investigate transmission dynamics, population structure and connectivity. Heterogeneous transmission patterns were observed across the region. A high proportion of monoclonal infections (67-100%), together with low nucleotide diversity (π: 0.0002-0.0008), suggests low transmission intensity or clonal expansion. Population structure analyses showed geographic structuring. Parasites from Amazonas-Peru were highly differentiated, while Brazil and Venezuela with Tumbes-Peru formed distinct clusters, indicating isolated transmission. While Ecuador clustered with Loreto-Peru, showed low differentiation from Colombia, French Guiana, Colombia and Honduras did not cluster with neighboring countries, suggesting limited regional connectivity. Identity-by-descent (IBD) analysis supported this pattern, identifying highly clonal populations (IBD >90%) within Peru, Venezuela, and Brazil and limited relatedness (IBD >10%) between Peru and other countries, consistent with local transmission and restricted gene flow. Our study shows complex population structure and connectivity of *P. vivax* across LATAM and highlight the utility of the Pv AmpliSeq Peru assay for regional molecular surveillance. Broader implementation will facilitate the identification of parasite transmission networks and importation routes, enabling early detection of parasite introductions and potential re-establishment of transmission in setting having achieved elimination.

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GENETIC DYNAMICS OF *PLAMODIUM FALCIPARUM* IN LORETO, PERU (2010 - 2012)

Jhon Zumaeta[1], Diana Cubas-Montecino[1], Gerardo Porras-Laymito[1], Stella M. Chenet[2], Ananias A. Escalante[3], Dionicia Gamboa[1], Luis Cabrera-Sosa[1], Irene Delgado de la Flor[1]

[1]Laboratorio de Malaria: Parásitos y Vectores, Facultad de Ciencias e Ingeniería, Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Instituto de Investigación de Enfermedades Tropicales, Universidad Nacional Toribio Rodriguez de Mendoza de Amazonas, Chachapoyas, Peru, [3]Department of Biology, Institute for Genomics and Evolutionary Medicine,Temple University, Philadelphia, PA, United States

Malaria remains a major public health concern in Peru despite the multiple control efforts like the Malaria Control in Andean Border Areas program (PAMAFRO). In the Peruvian Amazon, *Plamodium falciparum* (Pf) populations have shown shifts in their genetic profiles before and after intervention campaigns. However, their genetic dynamics in Loreto, the main region in the Peruvian Amazon, remain poorly characterized after the PAMAFRO program. Previous molecular surveillance has improved our understanding of parasite establishments and transmission. Our study aims to describe the genetic dynamic of Pf in Loreto between 2010-2012, immediately after PAMAFRO program. Using 11 neutral microsatellite markers, we analysed genetic diversity and population structure in 79 Pf samples collected across multiple localities in Loreto; additionally, dhfr/dhps resistance-associated polymorphisms were genotyped to evaluate the antifolate resistance haplotypes. Our results reveal heterogeneous genetic diversity across sites and a gradual decline in diversity over time (He: 0.53-

0.26). Very low differentiation was observed among several localities in Loreto (pairwise Fst: 0-0.04). STRUCTURE analyses suggest the presence of two to three genetic clusters, with a shift in cluster composition in 2011. At K = 3, a cluster from 2010 becomes more predominant in 2011, but is nearly absent by 2012, indicating a temporal shift in population structure. DAPC analysis supports this pattern, revealing clear genetic clusterization by year: 2010 and 2012 appear in genetically distinct clusters, with 2011 as a transitional phase. Additionally, dhfr/dhps haplotypes showed high diversity in 2010, but then declining over time, culminating in the fixation of the RICNI-SGEGA haplotype in 2012. Overall, Pf populations in Loreto after PAMAFRO showed marked temporal genetic changes followed by haplotype fixation. These findings highlight the importance of molecular studies in years when *P. falciparum* transmission was not adequately tracked, as they provide retrospective insight into the dynamics of parasite populations during those periods.

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IMPACT OF HELMINTH COINFECTION ON *PLASMODIUM FALCIPARUM* VACCINE TARGET DIVERSITY IN MFOU, CAMEROON

Francis ZEUKENG[1], Honore Awanakam Awanakam[1], Loic Jimmy Tekendong Kemo[1], Belta Atabonglefack Fotabong[1], Raymond Ngomdjum Tabue[2], Esemu Livo Forgu[1], Chedjou Kengne Jean-Paul[1], Khadim Gueye[3], Agnenga Balonga Anie Frandzine[1], Tappe Kamgmo Ange[1], Tiani Lionel Ulrich[1], Djounda Dieffouo Romeo Brice[1], Gwanmesia Philomina[1], Francine Ntoumi[4], Numfor Nkah Leonard[1], Veronique Penlap Beng[1], Wilfred Fon Mbacham[5], Rose Gana Fomban Leke[5], Stephen Mbigha Ghogomu[6], Ayong Lawrence[7], Lucas Amenga-Etego[8], Jude Bigoga Daiga[1]

[1]The Biotechnology Center, University of Yaoundé I, Yaoundé, Cameroon, [2]Vector Control Unit, National Malaria Control Program (NMCP), Yaoundé, Cameroon, [3]African Bioinformatics Center for Omics (ABCOMICS, Dakar, Senegal, [4]Fondation Congolaise pour la Recherche Médicale, Brazzaville, Republic of the Congo, [5]The Biotechnology Centre, University of Yaounde I, Yaounde, Cameroon, [6]Department of Biochemistry and Molecular Biology, Faculty of Science, University of Buea, Buea, Cameroon, [7]Centre Pasteur du Cameroun, Yaoundé, Cameroon, [8]West African Centre for Cell Biology of Infectious Pathogens (WACCBIP), College of Basic and Applied Sciences, University of Ghana, Accra, Ghana

Malaria morbidity frequently overlaps with povertyrelated infections such as soiltransmitted helminths (STHs), which may compromise malaria vaccine efficacy. This study assessed the impact of STH comorbidity on the genetic stability of key *Plasmodium falciparum* antigens, including the circumsporozoite protein (*pfcsp*) targeted by RTS,S/AS01 and R21/MatrixM vaccines, and the merozoite surface protein genes *pfmsp1* and *pfmsp2*, which encode surface antigens associated with parasite diversity and multiplicity of infection. A total of 523 participants were screened in the Mfou Health District for malaria and STHs using recommended protocols, and the genetic diversity of *pfcsp*, *pfmsp1*, and *pfmsp2* was analysed in monoinfected and coinfected isolates. Fulllength *pfcsp* sequences were generated using nanopore sequencing (ONT). Withinhost parasite diversity and population structure differentiation between mono and coinfected groups were quantified using Hudson's fixation index (FST). Malaria prevalence was 31.36% (164/523) by PCR, with *P. falciparum* (93.94%) as the dominant species. STH infections—mainly *Ascaris lumbricoides* and *Trichuris trichiura*—were detected in 10.71% (56/523), with 3.63% (19/523) coinfected with *P. falciparum*. PCoA and MDS revealed distinct population structures between mono and coinfected isolates, with moderate genetic differentiation (mean FST = 0.254) based on *pfcsp* sequences. Monoinfected parasites exhibited greater diversity (209 mutations, 205 indels, 43 haplotypes) than coinfected ones (56 mutations, 19 indels, 22 haplotypes), with most variants located in the *pfcsp* Cterminal region. Variation in NANP repeat numbers was observed, including a newly identified motif. Eighteen nonsynonymous mutations were detected in the Th2R and Th3R regions among monoinfected parasites, while no coding mutations were found in coinfected individuals. These patterns suggest that STH coinfection likely influences the genetic landscape of *P. falciparum* and underscore the need to consider comorbidities and local parasite genetic diversity when assessing malaria vaccine efficacy.

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ESTABLISHING ROBUST AND ACCESSIBLE BIOINFORMATICS TOOLS FOR MALARIA GENOMIC DATA ANALYSIS ON THE TERRA CLOUD-BASED PLATFORM

Gerald Mboowa[1], Kathryn Murie[2], Bernd Bohmeier[3], Jonathan Smith[1], Stephen Schaffner[1], Bassirou Ngom[4], Katherine DeRuff[1], Margaret Laws[5], Angela Early[1], Daniel J. Park[1], Daouda Ndiaye[4], Sarah K. Volkman[5], Jason A. Hendry[3], Bryan Greenhouse[2], Daniel E. Neafsey[5], Bronwyn MacInnis[1]

[1]Broad Institute, Cambridge, MA, United States, [2]EPPIcenter, Division of HIV, ID, and Global Medicine, University of California San Francisco, San Francisco, CA, United States, [3]Max Planck Institute for Infection Biology, Berlin, Germany, [4]International Research Training Center on Genomics and Health Surveillance (CIGASS), Cheikh Anta Diop University, Dakar, Senegal, [5]Department of Immunology and Infectious Diseases, Harvard T. H. Chan School of Public Health, Boston, MA, United States

Bioinformatic analysis capacity remains a critical barrier to fully realising the potential of malaria genomic surveillance, with major bottlenecks arising from restricted access to computational infrastructure and limited specialised programming expertise. Here, we advance accessible, validated genomic analysis for key Plasmodium public health applications by implementing robust, ready-to-use workflows in Terra—a secure, user-friendly, open-access, cloud-based data analysis platform for the global community. We describe the adaptation and implementation of two previously established bioinformatic pipelines for processing Plasmodium amplicon sequencing data: Mad4Hatter for short read Illumina data and Nomadic for long read ONT data. Originally developed to run locally or on High-Performance Computing clusters, we adapted these pipelines to run efficiently on the Terra cloud platform, providing robust and user-friendly analyses that are independent of local compute resources or advanced computational expertise. Key outputs include drug resistance marker profiles, with Mad4Hatter providing microhaplotype level information to support downstream applications such as molecular correction in therapeutic efficacy studies. We emphasise the advantages of working in a secure cloud environment, highlighting the practical considerations such as cost and analysis turnaround time, as well as safeguards that protect sensitive data while enabling access-controlled sharing to support collaboration. Furthermore, we provide robust, standardised workflows that produce detailed summaries for assessing sequencing data quality. These workflows are part of a larger data analysis ecosystem designed to deliver accessible, reliable tools and support for the malaria genomics community. We also show the application of these tools for drug resistance marker genotyping, including local workflow development, data analysis, and NMCP reporting, and address practical considerations for deploying these tools and the Terra platform across the malaria genomics community.

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IDENTIFICATION OF FAS AND FASL-REACTIVE PROTEINS IN *PLASMODIUM FALCIPARUM* AND THEIR POTENTIAL ROLE IN PROGRAMMED CELL DEATH

Lina Margarita Solis[1], Ricardo Correa[2], Carmenza Spadafora[3]

[1]INDICASAT-AIP, Panama, Panama, [2]Instituto de Investigaciones Científicas y Servicios de Alta Tecnología, Panama, Panama, [3]Carmenza Spadafora, Panama City, Panama

Programmed cell death (PCD) signaling pathways in *Plasmodium falciparum* remain to be fully elucidated. Understanding these mechanisms is critical for identifying novel parasite death pathways. Building on our previous findings of FAS and FASL-associated signaling in infected red blood cells (iRBCs), we investigated the identity and spatial organization of parasite-derived components recognized by anti-human FAS and FASL antibodies. Immunofluorescence microscopy revealed a discrete distribution of parasite structures exhibiting FAS and FASL reactivity. These signals showed strong

spatial colocalization (Pearson's r = 0.84) and asymmetric overlap (Manders' coefficients tM1 = 0.906, tM2 = 0.680). To identify the proteins associated with these signals, immunoprecipitation was performed using anti-human FAS and FASL antibodies, followed by SDS-PAGE separation and LC-MS-MS analysis. BLASTp alignment against the *P. falciparum* 3D7 proteome identified several parasite-encoded proteins, including PF3D7_0321700, PF3D7_0818200 (14-3-3 protein), and PF3D7_0318100 (stomatin-like protein), consistently associated with the immunoprecipitated fractions. To confirm parasite origin, corresponding gene sequences were analyzed via BLASTn against the human genome, revealing no significant homology. Together, these findings provide convergent molecular and spatial evidence for a FAS and FASL-like signaling framework in *Plasmodium falciparum*, advancing the mechanistic understanding of apoptosis-like pathways in this parasite.

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THE GENOME-WIDE LANDSCAPE OF MUTATION AND RECOMBINATION IN MALARIA PARASITE *PLASMODIUM FALCIPARUM* GENETIC CROSSES

Xue Li[1], Shalini Nair[1], Grace Arya[1], Marina McDew-White[1], Lisa Checkley[2], Sudhir Kumar[3], Francois Nosten[4], Stefan Kappe[3], Ian Cheeseman[1], Ashley Vaughan[3], Michael Ferdig[5], Timothy Anderson[1]

[1]Texas Biomedical Research Institute, San Antonio, TX, United States, [2]University of Notre Dame, Notre Dame, IN, United States, [3]Seattle Children's Research Institute, Seattle, WA, United States, [4]Shoklo Malaria Research Unit, Mae Sot, Thailand, [5]Univ of Notre Dame, Dept of Biology, Notre Dame, IN, United States

Genetic diversity in *Plasmodium falciparum* arises through mutation and recombination, processes that shape parasite adaptation and evolution. However, the genome-wide patterns of mutation, recombination, and structural variation remain incompletely characterized. Here we analyzed more than 2,000 cloned progeny from three experimental genetic crosses, including both F1 and F2 generations, to characterize how recombination and mutation impact genomic diversity in *P. falciparum*. We combined telomere-to-telomere de novo genome assemblies of parental strains with deep sequencing of thousands of recombinant progeny, enabling high-resolution analysis of genome inheritance patterns. Using these data, we estimated de novo mutation rates for single nucleotide polymorphisms (SNPs), indels, and large structural variants, and compared their distribution with the genome-wide recombination landscape. We characterized the genome-wide distribution and inheritance of structural variation across recombinant progeny and evaluated its relationship with recombination patterns. We further quantified the contribution of structural variants to genomic divergence relative to point mutations. In addition, direct observation of de novo mutations revealed context-dependent mutational processes associated with local genome architecture. Together, these results provide a comprehensive genome-wide view of the mutational and recombination landscape of *P. falciparum*.

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GENETIC HETEROGENEITY OF *PLASMODIUM FALCIPARUM* ACROSS AREAS OF VARYING TRANSMISSION INTENSITY IN ETHIOPIA BASED ON MEROZOITE SURFACE PROTEIN (MSP) *MSP1* AND *MSP2* GENES

Abeba Gebretsadik Reda[1], Ashenafi Aseffa[1], Lemu Golassa[2], Hassen Mamo[3]

[1]Ethiopian Public Health Institute, Addis Ababa, Ethiopia, [2]Addis Ababa University, Addis Ababa, United States, [3]Addis Ababa University, Addis Ababa, Ethiopia

Background: The genetic heterogeneity of *Plasmodium falciparum* informs targeted interventions and assesses the effectiveness of these interventions. This study helps assess the genetic heterogeneity of *Plasmodium falciparum* associated with different malaria transmission areas in Ethiopia. Methods: The study was a health facility-based cross-sectional study conducted between October 2015 and November 2019. Finger-prick and dried blood samples were collected from febrile patients. Individuals were selected from three malaria-endemic areas with different malaria transmission intensities. Microscopy, RDT, and QPCR were used for screening. Nested PCR was performed to genotype *P. falciparum* using the *msp1* and *msp2* genes. Results: Among the 661 participants, 347 (52.5%) were male, and 314 (47.5%) were female. Of these, 150 samples were successfully genotyped. Moving to genotyping outcomes, the proportions of *msp1* and *msp2* were 45.3% and 43.3%, respectively, across the sites. Notably, Metema exhibited the highest complexity of infections, with mean MOI values of 1.53 for *msp1* and 2.14 for *msp2* gene. Heterozygosity also showed notable changes, with values of 0.65 for msp1 and 0.70 for *msp2* in 2015. By 2019, these values decreased to 0.60 for *msp1* and 0.65 for *msp2,* indicating a reduction in genetic diversity. Collectively, these findings highlight temporal variability in allele frequencies, multiple infections, and heterozygosity between 2015 and 2019, suggesting shifts in the *P. falciparum* population dynamics in Metehara. Conclusions: Taken together, these results indicate that genetic heterogeneity has the potential to provide important complementary information to optimize malaria control interventions.

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TRANSCRIPTOMIC PROFILING OF SEVERE *PLASMODIUM FALCIPARUM* MALARIA WITH PROSTRATION IN UGANDAN CHILDREN

Bryan Evan Cummings[1], Emily Stucke[1], Jonathan Lawton[1], Sobota Rafal[2], Jeanpaul Courneya[1], Isaac Otolia[3], Ruth Ajilong[3], Joseph Owori[3], Miriam Baluka[3], Matthew B. Laurens[1], Pauline Byakika-Kibwika[4], Mark A. Travassos[1]

[1]University of Maryland School of Medicine, Baltimore, MD, United States, [2]Northwestern University School of Medicine, Chicago, IL, United States, [3]Makerere University, Kampala, Uganda, [4]Mbarara University of Science and Technology, Mbarara, Uganda

Despite advances in treatment and prevention, malaria continues to cause significant pediatric morbidity and mortality worldwide, particularly in sub-Saharan Africa, where *Plasmodium falciparum* predominates. *P. falciparum*, the most virulent human malaria parasite, is responsible for most severe disease manifestations, including prostration (profound weakness), which is one of the most common presentations of severe malarial disease. This study investigated whether the prostration phenotype possesses a unique transcriptomic profile among severe malaria syndromes and if prostration with central nervous system involvement is distinct from isolated prostration. We performed targeted RNA capture followed by RNA sequencing on *P. falciparum* isolates from Ugandan children aged 6-months to 14 years with severe malaria enrolled between 2023 and 2025. Prior to analysis, variant surface antigens belonging to the *var*, *rif*, and *stevor* multigene families were removed due to high sequence variability and mapping limitations. Differential gene expression analyses inclusive of 5,020 genes compared cases with prostration (n = 12) to those without (n = 8), and cases with prostration plus CNS involvement (n = 8) versus isolated prostration (n = 12). Overall, comparisons of cases with prostration to those without yielded no differentially expressed parasite genes. Similarly, analysis of prostration alone versus prostration with CNS involvement revealed only a single differentially expressed gene (FDR = 0.000006), belonging to the *phist* multigene family, which we have previously linked to processes that escalate disease severity, including host erythrocyte remodeling and parasite cytoadherence. These findings represent the first attempt to define a specific transcriptomic profile of the prostration phenotype and suggest that parasite gene expression patterns among severe malaria syndromes may be broadly similar and distinct from those associated with uncomplicated malaria. Results inform the design of future studies seeking to characterize parasite contributions to individual severe malaria phenotypes.

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A POPULATION-GENETIC MODEL FOR THE SPREAD OF *PLASMODIUM FALCIPARUM* PARASITES WITH *PF*HRP2/3 DELETIONS

Loyce Kayanula[1], Kanika Verma[2], Qiuying Cheng[3], Praveen Bharti[4], Douglas Jay Perkins[3], Kristan Alexander Schneider[5]

[1]Technical University Chemnitz, Chemnitz, Germany, [2]New Delhi, DL, India, [3]University of New Mexico, Albuquerque, NM, United States, [4]Jabalpur, India, [5]UNM, Albuquerque, NM, United States

The WHO prioritizes surveillance of *Pf*hrp2/3-deleted *Plasmodium falciparum* parasites, which evade detection by HRP2-based RDTs. Because these deletions predate widespread RDT deployment, diagnostic escape cannot explain their origin. *Pf*HRP2 also acts as a virulence-associated inflammatory factor, and infections with *Pf*hrp2/3-deleted parasites are associated with less symptomatic disease, suggesting weak immune evasion. Reduced parasite growth due to loss of *Pf*HRP2-mediated heme detoxification during hemoglobin digestion may prolong asymptomatic or submicroscopic infections. To investigate mechanisms underlying the spread of *Pf*hrp2/3-deleted parasites, we developed a population-genetic model that assumes weak immune and diagnostic evasion, leading to asymptomatic or submicroscopic infections and false-negative RDTs. The deterministic model follows haplotype frequencies across discrete generations of transmission cycles and incorporates multiplicity of infection (MOI), determining co-occurrence of deletion and wild-type parasites within hosts, modulating immune responses, and diagnostic sensitivity. Using hypothetical selection parameters, we simulated the spread of *Pf*hrp2/3 deletions and compared it with the evolution of drug resistance. Our model mechanistically shows how MOI influences deletion dynamics. Unlike drug resistance evolution, which is relatively insensitive to MOI, high MOI slows the early spread of deletion haplotypes. When mixed infections are common, selective advantages are reduced because deleted parasites frequently co-occur with wild-type parasites. This delays the spread of *Pf*hrp2/3 deletions relative to drug resistance, even under identical selection pressures. High MOI delays the early spread of *Pf*hrp2/3 deletions, thereby weakening detectable signatures of selection. Combined with weak and indirect selection pressures and high mutation rates in subtelomeric regions containing *Pf*hrp2/3, this can obscure empirical signals of selection. Controlled *in vitro* experiments are therefore needed to clarify the selective environment favoring these deletions.

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PLASMODIUM FALCIPARUM PANGENOME IMPROVES GENOTYPING SENSITIVITY

Ankit Dwivedi[1], **Yiwei Yang**[1], Kara Moser[1], Olukemi Ifeonu[1], Chakshu Gandhi[1], Emily M. Stucke[2], Drissa Coulibaly[3], Ogobara K. Doumbo[3], Mahamadou A. Thera[3], Chanthap Lon[4], David L. Saunders[5], Asha Patil[6], Ashley Vaughan[7], B. Kim Lee Sim[6], Myaing M. Nyunt[2], Miriam K. Laufer[2], Mark A. Travassos[2], Shannon Takala-Harrison[2], Christopher V. Plowe[2], Joana C. Silva[1]

[1]Institute for Genome Sciences, University of Maryland School of Medicine, Baltimore, MD, United States, [2]Malaria Research Program, Center for Vaccine Development & Global Health, University of Maryland School of Medicine, Baltimore, MD, United States, [3]Parasites and Microbes Research and Training Center, University of Sciences, Techniques and Technologies, Bamako, Mali, [4]International Center of Excellence in Research, National Institute of Allergy and Infectious Disease, National Institutes of Health, Phnom Penh, Cambodia and Laboratory of Malaria and Vector Research, NIH, Bethesda, MD, United States, [5]Armed Forces Research Institute of Medical Sciences, Phnom Penh, Cambodia, [6]Sanaria Inc., Rockville, MD, United States, [7]Center for Global Infectious Disease Research, Seattle Children's Research Institute, Seattle, WA, United States

The *Plasmodium falciparum* (*Pf*) 3D7 genome, published in 2002, was derived from a West African lab-cultured strain and is the canonical reference genome for malaria research. Although the *Pf* core genome is largely syntenic across strains from different geographic regions, by using different *Pf* genome references we have observed ~2100 structural variants (SVs, >50bp) among strains from Africa and Southeast Asia, with at least ~300 private SVs in each region. These findings highlight a key limitation of using a single linear reference genome, particularly for the genotyping of genomic segments absent or re-arranged in the reference assembly. We hypothesized that the use of a reference *Pf* pangenome, by enabling a more comprehensive genotyping of strains using a common set of coordinates, would reveal new genomic segments not present in Pf3D7 and enable estimation of mutation frequencies in different geographic regions. To this end, we constructed a *Pf* pangenome using a graph-based framework and 20 high-quality, long read-based genome assemblies from West (Mali, Guinea) and East Africa (Malawi), Southeast Asia (Myanmar, Cambodia, Laos) and South America (Brazil). The resulting graph encodes ~54.5 Mb of non-redundant sequence across ~2.27 million nodes, reflecting accessory and divergent sequences relative to Pf3D7. This *Pf* pangenome enabled more comprehensive identification of variants, revealing ~76K accessory SNPs and ~3500 accessory SVs among the 20 strains from different regions. These accessory mutations localize to sequences missing in Pf3D7 and are therefore not discoverable when using only Pf3D7 as the reference. Ongoing research includes the characterization of SVs fixed for distinct haplotypes in different geographic regions to understand potential functional implications. Overall, we have demonstrated that the use of a *Pf* pangenome improves the sensitivity of SNP and complex genomic variant detection and reduces the reference bias in *Pf* genomic analyses.

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MOLECULAR CHARACTERIZATION OF *PLASMODIUM KNOWLESI* FROM MACAQUES ON BELITUNG ISLAND, INDONESIA, AN AREA NON-ENDEMIC FOR HUMAN MALARIA

Matthew R. Kulpa[1], Irina Diekmann[1], Taniawati Supali[2], Abakar Gankpala[1], Rahmat Alfian[2], Yossi Destani[2], Elisa Iskandar[2], Noviani Sugianto[2], Balbir Singh[3], Paul C.S. Davis[3], Peter R. Fischer[1]

[1]Infectious Diseases Division, Department of Medicine, Washington University School of Medicine, Saint Louis, MO, United States, [2]Department of Parasitology, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia, [3]Universiti Malaysia Sarawak (UNIMAS) Malaria Research Centre, Kota Samarahan, Malaysia

Plasmodium knowlesi has emerged as a prominent cause of human malaria in southwest Asia over the past two decades. Unlike the other four *Plasmodium* species that cause human malaria, *P. knowlesi* is the only zoonotic species known to be naturally maintained in macaque (*Macaca fascicularis*) hosts. To better understand this increasing prevalence of *P. knowlesi* infections, blood was collected from free-ranging macaques in Belitung District, Bangka Belitung Islands Province in western Indonesia. The district is considered to be free from human malaria transmission. *P. knowlesi* has been reported previously in humans, macaques, and *Anopheles* vectors from Sumatra, Kalimantan and other islands in western Indonesia but not from Belitung. In total 163 blood samples were screened by qPCR for various *Plasmodium* species, including *P. knowlesi*. Of the 130 macaques that tested positive (79.8%) for any *Plasmodium* infection, *P. inui* was most prevalent (41.7%), followed by *P. knowlesi* (24.5%), *P. coatneyi* (24.5%), and *P. cynomolgi* (13.5%). Co-infections involving more than one *Plasmodium* species were frequent, though no samples comprised all four species simultaneously. In addition, the complete mitochondrial genome of 6 *P. knowlesi* isolates were sequenced (6,132 - 6,155 bp) and annotated with 34 ribosomal RNA fragments and 3 protein coding DNA sequences. Pairwise distance analysis showed on average that our *P. knowlesi* mitochondrial sequences were more like other *P. knowlesi* sequences from macaque hosts in the Narathiwat province of southern Thailand (0.11%) compared to those from the Sarawak and Sabah states of Malaysia (0.15%). However, little genetic difference was seen when comparing between our sequences and other *P. knowlesi* sequences originating from either human (0.14%) or macaque hosts (0.15%) regardless of geography. Our molecular evidence confirms the presence of zoonotic *P. knowlesi* and other *Plasmodium* species in macaques from Belitung. Moreover, there

appears to be little genetic discrepancy between macaque and human strains of *P. knowlesi*, emphasizing the risk of human transmission and need for increased surveillance.

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WITHIN-HOST AMINO ACID DIVERSITY IN BLOOD-STAGE *PLASMODIUM FALCIPARUM* AMA-1 AND CSP REVEALS EPITOPE-LEVEL IMMUNE SELECTION

Peninnah Winnie Makusa[1], Christine F. Markwalter[2], Abdirahman Abdi[3], Wendy P. O'Meara[4], Francis M. Ndungu[3], Steve M. Taylor[4]

[1]KEMRI-Wellcome Trust Research Program, Kilifi, Kenya, [2]Duke Global Health Institute, Durham, NC, United States, [3]KEMRI-Wellcome Trust Research Programme, Kilifi, Kenya, [4]Duke University, Durham, NC, United States

RTS,S/AS01 and R21/Matrix-M are partially effective, in part due to the extensive antigenic diversity characteristic of malaria proteins. The host immune system exerts pressure on *Plasmodium falciparum* within individual hosts. Although population-level analyses can detect such signatures of selection, they capture aggregate signals across hosts and vectors and may miss immune-driven selection occurring within individual infections. We investigated within-host *P. falciparum* blood-stage amino acid diversity at AMA1 domain I and the CSP Cterminal region using high-depth amplicon sequencing data from 223 naturally infected individuals (≥ 1 year) in western Kenya followed for 14 months. Observed diversity was compared to a null distribution generated from 1000 simulated datasets. Most infections were polyclonal (62% for AMA-1; 68% for CSP). Compared to expected, observed within-host diversity was higher within the AMA-1 domain I (median observed–expected 0.185, 95% CI 0.040–0.324, P = 0.013) and the AMA-1 C1L B cell epitope (0.133, 0.071–0.198, P < 0.0001), but did not differ from expected in the CSP C-terminal region (0.081, -0.027–0.091, P = 0.16). At the epitope level, within-host diversity was higher than expected at the CSP-DV10 B cell epitope (0.137, 0.099–0.170, P < 0.0001) and lower than expected at the CSP-Th3R $CD8^+$ T cell epitope (-0.149, -0.200–-0.101, P < 0.0001). At CSP-Th2R $CD4^+$ T cell epitope, observed within host amino acid diversity did not differ from expected (0.055, -0.007–0.104, P = 0.081), except in symptomatic infections (0.195, 0.062–0.344, P = 0.007). At the amino acid level, within-host diversity matched the expected, consistent with host immune pressure operating at the epitope rather than the residue level. The contrasting patterns of within-host diversity compared to expected within AMA-1 domain I and the CSP C-terminal region, and across their B- and T-cell epitopes, suggest differences in antigen expression timing and immune pressure. These findings support assessing *P. falciparum* diversity at the within-host scale and suggest that epitope-level diversity may be relevant for malaria vaccine design.

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DISCRIMINATING IMPORTED *PLASMODIUM VIVAX* INFECTIONS USING WHOLE-GENOME SEQUENCING IN THE PERUVIAN AMAZON

Mahdi Safarpour[1], Luis Cabrera-Sosa[2], Johanna Helena Kattenberg[3], Ramses Deyaert[3], Anna Rosanas-Urgell[3], Joseph M. Vinetz[4], Dionicia Gamboa[2], Jean-Pierre Van geertruyden[1], **Christopher Delgado Ratto**[1]

[1]University of Antwerp, Antwerp, Belgium, [2]Universidad Peruana Cayetano Heredia, Lima, Peru, [3]Institute of Tropical Medicine, Antwerp, Belgium, [4]Yale University, New Haven, CT, United States

In areas approaching malaria elimination, identifying and characterizing imported infections is critical, as they can reintroduce parasites and sustain residual transmission. Human mobility plays a key role in sustaining malaria transmission by introducing parasites into receptive communities. Genomic approaches offer the potential to characterize parasite populations across locations and support the identification of imported infections.We analyzed 54 confirmed *Plasmodium vivax* infections from three communities in the Peruvian Amazon (Libertad, Gamitanacocha, and Urcomiraño), including 15 individuals reporting recent travel. Whole-genome sequencing (WGS) was performed to generate high-resolution genomic data. To discriminate imported from local infections, we applied principal component analysis (PCA), ADMIXTURE analysis, identity-by-descent (IBD) estimation, and neighbor-joining phylogenetic trees.Parasite populations from Libertad and Gamitanacocha exhibited higher genetic diversity, whereas Urcomiraño showed lower diversity and greater genetic differentiation, suggesting partial isolation. PCA and ADMIXTURE analyses supported this structure, with clear separation of Urcomiraño and greater admixture in Libertad. IBD analyses revealed high relatedness at lower thresholds, with more distinct clusters at higher thresholds. Nucleotide diversity was higher among travelers in Libertad, but similar between groups in Urcomiraño. Only 2 of 15 traveler infections were clearly classified as imported based on genomic data. In most cases, self-reported travel history did not concord with genetic clustering.WGS provides detailed insights into parasite diversity and connectivity in low-transmission settings. However, in clonal or highly related populations, genomic data alone may have limited resolution to distinguish imported from local infections. Integrating genomic, epidemiological, and mobility data is therefore critical for accurate classification of infection origin.

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TRANSCRIPTIONAL SIGNATURES OF RECOVERY IN CHILDREN WITH SEVERE MALARIAL ANEMIA

Jackson Ochieng[1], Shamim Osata[2], Shirley Achieng[2], Clinton O. Onyango[3], Tyrus Omondi[4], Collins Ouma[2], Philip Seidenberg[5], Kristan Schneider[5], Qiuying Cheng[5], Ivy Hurwitz[5], Samuel B. Anyona[2], Douglas Jay Perkins[5]

[1]Kenyatta University, Nairobi, Kenya, [2]Maseno University, Maseno, Kenya, [3]University of New Mexico - Kenya Global Health Programs, Kisumu, Kenya, [4]Masinde Muliro University of Science and Technology, Kakamega, Kenya, [5]University of New Mexico, Albuquerque, NM, United States

Severe malarial anemia [SMA, hemoglobin (Hb)<6.0 g/dL] is the primary clinical manifestation of severe malaria in children under 5 years living in *Plasmodium falciparum* holoendemic transmission areas. To characterize the molecular response underlying post-treatment recovery in SMA, whole-blood transcriptomes were generated from 25 paired samples collected at acute infection (day 0, pre-treatment) and on follow-up (day 14, post-treatment) in Siaya County, western Kenya. Differential gene expression analysis using edgeR revealed 6,932 differentially expressed genes [DEGs, 3,894 up- and 3,038 down-regulated; false discovery rate - adjusted *p*-value (*q*-value)<0.05] between day 0 and 14. Network analysis (MetaCore™) linked down-regulated DEGs to the *E2F1↔TCF7L2 (TCF4)↔SRF↔PPAR-gamma↔CUX1* process network and up-regulated DEGs to the *FOXP3↔EZH2↔SUZ12↔PKA-cat (cAMP-dependent)↔ATM* process network. The top enriched pathway was the Signal Transduction Calcium-mediated Signaling (*q*-value=7.852E-12), which is essential for normal physiological processes. Modular analysis (BloodGen3Module) showed enrichment of up-regulated modules related to lymphocytes, T cells, prostanoids, gene transcription, protein synthesis, and modification functions. Down-regulated modules were enriched for inflammation, neutrophils, neutrophil activation, cytokines/chemokines, and monocyte functions. Functional enrichment analyses of DEGs (*q*-value<0.05) using clusterProfiler converged at allosteric regulation of the cytoskeleton, innate immune activation, inflammatory response to external stimuli and secretory trafficking, and immune signaling. The MAPK signaling pathway and IgSF CAM signaling (GeneRatio=0.046) were the top-enriched biological pathways. Collectively, these findings define the transcriptional landscape of SMA recovery and provide insight into the mechanisms underlying disease resolution.

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STRUCTURAL AND EXPORTED TRANSCRIPTOMIC SIGNATURES DRIVE HOST ERYTHROCYTE REMODELING IN SEVERE MALARIA ANEMIA

Sarah Naulikha Kituyi[1], Qiuying Cheng[2], Clinton Odhiambo Onyango[2], Ivy Hurwitz[3], Philip Seidenberg[2], Kristan Schneider[4], Christophe Lambert[2], Benjamin McMahon[5], Ananias A. Escalante[6], Samuel Bonuke Anyona[7], Douglas Jay Perkins[2]

[1]Mount Kenya University, Thika, Kenya, [2]University of New Mexico, Albuquerque, NM, United States, [3]Univ of New Mexico, Albuquerque, NM, United States, [4]UNM, Albuquerque, NM, United States, [5]Los Alamos National Laboratory, Los Alamos, NM, United States, [6]Temple University, Philadelphia, PA, United States, [7]Maseno University, Kisumu, Kenya

Severe malaria anemia (SMA; Hb < 6.0 g/dL, any parasite density) is driven by extensive host erythrocyte remodeling and remains a leading cause of morbidity and mortality in children under five in holoendemic *Plasmodium falciparum* transmission regions. Although clinical severity parameters are well-defined, the specific parasite transcriptional adaptations that contribute to severe disease remain incompletely understood. We investigated parasite transcripts upregulated in SMA compared to Non-SMA (Hb > 6.0 g/dL) to identify potential drivers of pathogenesis, analyzing RNA-seq data from 60 Kenyan children (3-36 months) with *P. falciparum* infection [SMA (n=20) and Non-SMA (n=40)]. Differential gene expression analysis identified multiple transcripts significantly upregulated in children with SMA (Log_2FC > 1 and *P*-value < 0.05). Specifically, we identified structural components of the parasite's cytoskeleton, including the gamma-tubulin complex component (*Pf*KE01_090038100, Log_2FC = 1.63) and tubulin gamma chain (*Pf*KE01_080009500, Log_2FC = 1.52). A membrane occupation and recognition nexus (MORN) repeat protein essential for the biogenesis of the inner membrane complex (IMC) (putative *Pf*KE01_050015700, Log_2FC = 1.14) was also significantly enriched. In addition, we identified upregulation of protein processing machinery, including type I signal peptidase (SPB) (*Pf*KE01_130026100, Log_2FC = 2.86) and serine/threonine protein kinase ARK2 (*Pf*KE01_030013600, Log_2FC = 1.68), which facilitate the export of virulence factors such as *Pf*EMP1 (*Pf*KE01_080014600, Log_2FC = 1.79). To maintain this high metabolic output, stress-response chaperones ClpB1 (*Pf*KE01_080022200, Log_2FC = 2.36) and HSP60 (*Pf*KE01_100020900, Log_2FC = 2.06) were significantly enriched. The results indicate that SMA is characterized by a distinct transcriptional profile of the *falciparum* parasite in the human bloodstream, favoring its shift toward accelerated asexual replication, increased virulence, and host remodeling. These findings have the potential to facilitate the identification of new therapeutic targets for severe disease outcomes.

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NETWORK BASED TRANSCRIPTOMIC ANALYSIS REVEALS METABOLIC STRESS MEDIATED IMMUNE DYSFUNCTION IN PEDIATRIC SEVERE MALARIAL ANEMIA

Tyrus O. Swaya[1], Samwel B. Anyona[2], Sharley A. Wasena[3], Shamim W. Osata[3], Jackson O. Ochieng[4], Qiuying Cheng[5], Ivy Hurwitz[5], Clinton O. Onyango[3], Philip D. Seidenberg[6], Kristan A. Schneider[7], Douglas J. Perkins[3]

[1]Masinde Muliro University of Science and Technology, Kakamega, Kenya, [2]Department of Medical Biochemistry, School of Medicine, Maseno University, Maseno, Kenya, Kisumu, Kenya, [3]University of New Mexico-Kenya Global Health Programs, Kisumu and Siaya, Kenya, Kisumu, Kenya, [4]Department of Biochemistry, Microbiology, and Biotechnology, School of Pure and Applied Sciences, Kenyatta University, Nairobi, Kenya, Nairobi, Kenya, [5]Center for Global Health, Department of Internal Medicine, University of New Mexico, Albuquerque, NM, USA, Albuquerque, NM, United States, [6]Department of Emergency Medicine, School of Medicine, University of New Mexico, Albuquerque, New Mexico, USA, Albuquerque, NM, United States, [7]Division of Translational Informatics Division, Department of Internal Medicine, University of New Mexico, Albuquerque, New Mexico, USA, Albuquerque, NM, United States

Severe malarial anemia [SMA, any density parasitemia with hemoglobin (Hb)<6.0 g/dL] remains a leading complication in *Plasmodium falciparum* (Pf) holoendemic regions. SMA is characterized by hypoglycemia and lactic acidosis, an alteration in carbohydrate metabolism. To define the transcriptomic profile of whole-blood gene expression, we analyzed samples from 66 Kenyan children, including those with severe malarial anemia (SMA; n = 25) and non-SMA controls (hemoglobin ≥ 6.0 g/dL; n = 41). Differential gene expression analysis was performed on 53,286 annotated transcripts using edgeR, applying a false discovery rate (FDR)-adjusted significance threshold of $padj \leq 0.050$. Functional enrichment analysis of significantly differentially expressed genes was conducted using MetaCore™. Among the 53,286 gene transcripts, 18,492 were assigned to carbohydrate metabolism-related gene networks based on annotations derived from the org.Hs.eg.db package. Of the 18,492 carbohydrate metabolism associated transcripts, 3,011 were differentially expressed between SMA and non-SMA groups, including 2,328 upregulated and 733 downregulated genes. Upregulated genes (e.g., *GATA1*↔*MECP2*↔*CTCF*↔*FBI-1*↔*CRY1*) were linked to enhanced glycolytic activity, epigenetic regulation of metabolic pathways, and circadian control of energy homeostasis. In contrast, downregulated genes (*STAT3*↔*SUZ12*↔*LHX2*↔*IRF1*↔H1F1A) suggested suppression of immune activation, chromatin remodeling, and hypoxia adaptation. GO enrichment analysis demonstrated enrichment of nuclear WNT/β-catenin signaling (*p*adj=3.55E-5) and suppression of CD40-dependent B-cell immune signaling (*p*adj=1.473E-11), representing the top dysregulated pathways in children with SMA. Additional GO enrichment analysis with Clusterprofiler revealed overrepresentation of autophagy and ubiquitin-mediated proteolysis with concurrent enrichment of endosomal-lysosomal compartments and ubiquitin-related mechanisms. Collectively, these alterations indicate disrupted energy homeostasis in SMA underlying disease pathogenesis.

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SPLENIC ACCUMULATION OF IMMATURE RETICULOCYTES AND *PLASMODIUM* SPECIES IN RHESUS MACAQUE MODELS

Bertin Vianou, Solomon Conteh, Min Su Cho, Junhui Duan, Jillian Neal, Tarik Ouahes, Sachy Orr Gonzalez, Lynn E. Lambert, Patrick Emmet Duffy

National Institutes of Health, NIAID/Lab of Malaria Immunology & Vaccinology, Bethesda, MD, United States

Traditionally, the spleen has been viewed as an immune surveillance organ to selectively filter and destroy senescent erythrocytes, infectious microorganisms and *Plasmodium*-infected erythrocytes (iEs). However, recent studies in asymptomatic infected adults have observed enriched levels of reticulocytes and non-phagocytosed *P. falciparum* and *P. vivax* iEs in the spleen. Intact, viable parasite densities were 100 to 1000 times higher in the spleen than peripheral blood for both species. Immature reticulocytes (CD71+) were also found colocalized in the same splenic compartments with *P. vivax* iEs of all asexual stages, suggesting a hidden endosplenic life cycle. Using Rhesus macaques inoculated with blood-stage *P. coatneyi* or *P. cynomolgi*, we 1) investigate splenic accumulation of immature reticulocytes and Plasmodium iEs occurs in nonhuman primates and 2) assess immune cell changes related to splenic parasite accumulation. Hematological parameters including immature reticulocyte proportion, parasite density, erythroid progenitors and parasite viability were analyzed in peripheral blood, bone marrow, and spleen samples of 10 Rhesus macaques (4 *P. coatneyi*-infected, 4 *P. cynomolgi*-infected, and 2 uninfected). Splenic accumulation of immature reticulocytes increased during *P. cynomolgi* infection (p=0.028) whereas P. coatneyi infection induced severe anemia and a delay in erythroid compensatory mechanisms. Spleen-to-circulating peripheral blood median parasite density ratios were higher during *P. coatneyi* than *P. cynomolgi* infections (3.62 vs 2.55). In both infections, all parasite stages were observed in the spleen, with

predominance of rings and trophozoites. The presence of iEs and immature reticulocytes in each spleen compartment (cords, sinus lumens, perifollicular zones, non-circulatory spaces) as well as splenic T cells, B cells and macrophage activation profile is now being assessed, and splenocytes will be characterized by immunophenotyping, cytokine/chemokine production and RNAseq. Results are expected to further elucidate the biology of an endosplenic asexual Plasmodium lifecycle.

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CO-EXPRESSION NETWORK ANALYSIS REVEALS DISTINCT TRANSCRIPTOMIC PATHWAYS IN CHILDREN WITH SEVERE MALARIAL ANEMIA AND SICKLE CELL ANEMIA

Shamim W. Osata[1], Clinton Odhiambo Onyango[2], Sharley A. Wasena[1], Qiuying Cheng[3], Ivy Hurwitz[4], Philip D. Seidenberg[3], Benjamin McMahon[5], Kristan Schneider[6], Collins Ouma[7], Samuel Anyona[4], Douglas Jay Perkins[3]

[1]Maseno University, Kisumu, Kenya, [2]Maseno University, Albuquerque, NM, United States, [3]University of New Mexico, Albuquerque, NM, United States, [4]Univ of New Mexico, Albuquerque, NM, United States, [5]Los Alamos National Laboratory, Los Alamos, NM, United States, [6]UNM, Albuquerque, NM, United States, [7]Maseno Univ, Kisumu, Kenya

Severe malarial anemia [SMA, hemoglobin (Hb) < 6.0 g/dL] and sickle cell anemia (SCA) remain major contributors to pediatric morbidity and mortality in holoendemic *Plasmodium falciparum* transmission regions such as western Kenya. Understanding the molecular landscape of children with SCA who develop SMA is critical for identifying novel therapeutic targets. To define the molecular landscape, whole-blood transcriptomes were characterized in children with SMA, stratified by sickle cell status into SCA (HbSS, n=7) and non-SCA (HbAA and HbAS, n=18). Participants were selected from an acute febrile cohort (2017-2024) at Siaya County Referral Hospital, Kenya. Weighted Gene Co-expression Network Analysis of 53,286 transcripts generated 27 modules, out of which four were significantly associated with SCA. When binary coding non-SCA/SCA as 0/1, modules M15 (p=5.66E-03), M19 (p=1.23E-02), and M24 (p=2.27E-02) showed positive correlations, and M23 (p=1.85E-03) displayed a negative association. Gene Ontology enrichment analysis identified modules associated with ribosome biogenesis and structural constituents of the ribosome (M15), innate immune activation of NAD^+ nucleosidase activity (M19), regulation of endocytosis and actin filament binding (M24), and macroautophagy and cytoskeletal organization (M23). Reactome pathway analysis across the significant modules further highlighted key biological processes, including RNA metabolism (M15, padj=3.54E-74), neutrophil degranulation (M19, padj=2.51E-29), immune system pathways (M24, padj=6.48E-09), and the MyD88-independent TLR4 cascade (M23, *p*adj=3.23E-04). Collectively, these results indicate dysregulation of ribosomal function, innate immune responses, endocytic processes, and autophagy in children with SCA who develop SMA, suggesting disruption of core cellular homeostatic mechanisms. These findings identify candidate pathways for further investigation and therapeutic development.

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PERIPHERAL BLOOD TRANSCRIPTOMIC PROFILING DIFFERENTIATES SEVERE MALARIAL ANEMIA FROM SEVERE ANEMIA

Qiuying Cheng[1], Clinton Odhiambo Onyango[2], Sharley A. Wasena[3], Shamim Wanjiku Osata[4], Samuel Anyona[5], Ivy Hurwitz[5], Sarah Naulikha Kituyi[6], Philip D. Seidenberg[1], Collins Ouma[7], Kristan Schneider[8], Benjamin McMahon[9], Christophe G. Lambert[1], Douglas Jay Perkins[1]

[1]University of New Mexico, Albuquerque, NM, United States, [2]Maseno University, Albuquerque, NM, United States, [3]Maseno University, Kisumu, Kenya, [4]University of New Mexico-Kenya Global Health Programs, Nairobi, Kenya, [5]Univ of New Mexico, Albuquerque, NM, United States, [6]University of New Mexico Center for Global Health, Embu, Kenya, [7]Maseno Univ, Kisumu, Kenya, [8]UNM, Albuquerque, NM, United States, [9]Los Alamos National Laboratory, Los Alamos, NM, United States

Severe malarial anemia (SMA: Hb<6.0 g/dL with any density parasitemia) is the primary clinical manifestation of severe malaria and a leading acute cause of morbidity and mortality in children under five in holoendemic *Plasmodium falciparum* transmission regions. In contrast, severe anemia without malaria (SA, Hb<6.0 g/dL) is typically chronic and also contributes substantially to morbidity and mortality in this population. To investigate SMA pathogenesis and disease outcomes, we performed transcriptomic analyses on peripheral blood collected from children (3-36 months) with SMA (n=25) or SA (n=20), matched by age, sex, and sickle cell status, enrolled in a prospective acute febrile cohort in Siaya County in western Kenya. Differential gene expression analysis uncovered 384 differentially expressed genes (160 up-regulated and 224 down-regulated) between the SMA and SA groups [FDR-adjust *P*-value (*Padj)*<0.05]. Transcripts for TNFα (Log_2FC=0.757), IL6 (Log_2FC=0.990), IL10 (Log_2FC=1.165), IFNG (Log_2FC=0.907), C1QA (Log_2FC=1.198), C1QB (Log_2FC=1.168), C1QC (Log_2FC=0.913), HMOX1 (Log_2FC=0.967), SLC39A8 (Log_2FC=1.091), LAG3 (Log_2FC=1.169) and HAVCR2 (Log_2FC=0.822) were up-regulated. In contrast, *MPO* (Log_2FC=-1.047), *ELANE* (Log_2FC=-0.915), and *AZU1* (Log_2FC=-1.192) were down-regulated. These patterns suggest that SMA is characterized by hemolysis-associated macrophage activation, complement induction, and mixed pro- and anti-inflammatory signaling, accompanied by upregulation of immune checkpoints and reduced neutrophil effector signatures. Additionally, SMA patients displayed transcriptomic features of metabolic collapse (elevated SLC2A6, Log_2FC=0.838) and severe tissue hypoxia (elevated EDN1 and ACE, Log_2FC=0.825 and 0.827, respectively). Up-regulation of *UBE2O* (Log_2FC=0.904), together with elevated *TNFα*, *IL6,* and *IFNG*, is consistent with ineffective erythropoiesis in SMA. Together, these results indicate that SMA is characterized by a profoundly dysregulated and imbalanced host response, distinguishing it from SA and providing new insight into the molecular basis of disease pathogenesis.

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TRANSCRIPTOME-WIDE DISEASE-DEPENDENT CIS-EQTL LANDSCAPE AND PATHWAY DYSREGULATION IN SEVERE MALARIAL ANEMIA

Clinton O. Onyango[1], Sharley A. Wasena[2], Shamim W. Osata[2], Qiuying Cheng[3], Ivy Hurwitz[3], Samuel Anyona[4], Collins Ouma[2], Philip D. Seidenberg[5], Christophe G. Lambert[6], Kristan Schneider[6], Benjamin McMahon[7], Douglas Jay Perkins[3]

[1]University of New Mexico-Kenya Global Health Programs, Kisumu and Siaya, Kenya, Albuquerque, NM, United States, [2]Department of Biomedical Sciences and Technology, School of Public Health and Community Development, Maseno University, Maseno, Kenya, [3]Center for Global Health, University of New Mexico, Albuquerque, NM, United States, [4]Department of Medical Biochemistry, School of Medicine, Maseno University, Maseno, Kenya, [5]Department of Emergency Medicine, University of New Mexico, Albuquerque, NM, United States, [6]Division of Translational Informatics, Department of Internal Medicine, University of New Mexico, Albuquerque, NM, United States, [7]Theoretical Biology and Biophysics Group, Theoretical Division, Los Alamos National Laboratory, Los Alamos, NM, United States

Severe malarial anemia (SMA: Hb<6.0 g/dL) is a life-threatening complication in children under five in *Plasmodium falciparum* malaria-endemic regions. Host genetic variation shapes clinical outcomes. To investigate host genetic determinants of SMA, we analyzed transcriptome-wide SNPs from RNA-seq data of 66 Kenyan children (3-36 months; 41 non-SMA, 25 SMA). Reads were aligned to GRCh38 using HISAT2 and processed with SAMtools and GATK. Variants were called using HaplotypeCaller (quality-filtered: QD<2, FS>30, MQ<40), yielding 5.7 million functionally annotated variants. After biallelic variant filtering (MAF≥0.05, MAC≥10), 126,684 high-confidence SNPs were retained. SNPs within 1Mb upstream of transcription start sites were assessed for disease-state-dependent genetic regulation using a cis-expression quantitative trait loci (cis-eQTL) genotype × disease interaction model, adjusting for

age, sex, and sickle-cell status. Interaction analysis identified 14,542 protein-coding genes with significant cis-regulatory effects. Variant density analysis identified the top five regulatory hotspots: *MBD3L4* (1,757 SNPs), *SSX4B* (1,619), *SMIM34* (1,554), *USP17* (1,392), and *USP20* (1,375). β-weighted pre-ranked gene set enrichment of interaction effects revealed significant (FDR-adjusted) SMA-dependent genetic enrichment of pathways involved in heme metabolism (Normalized Enrichment Score, NES=1.98), inflammatory response (NES=1.68), IL2/STAT5 signaling (NES=1.55), complement (NES=1.44), IL6/JAK/STAT3 signaling (NES=1.57), KRAS signaling (NES=1.39), and TNFα/NFκB signaling (NES = 1.40). In contrast, SMA was associated with reduced genetic regulation of MTORC1 signaling (NES=-1.70), G2M checkpoint (NES=-1.68), interferon alpha response (NES=-1.78), reactive oxygen species pathway (NES=-1.88), unfolded protein response (NES=-1.65), and glycolysis (NES=-1.44). Together, these findings reveal extensive cis-regulation in SMA, identify cis-regulatory variant clusters, and highlight both enhanced and diminished cis-eQTL modulation of erythropoiesis, inflammation, and immune signaling.

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UBIQUITIN PROTEASOME SYSTEM-MEDIATED MODULATION OF EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) SIGNALING IN KENYAN CHILDREN WITH SEVERE MALARIAL ANEMIA

Samuel Bonuke Anyona[1], Qiuying Cheng[2], Sharley A. Wasena[3], Shamim W. Osata[4], Ivy Hurwitz[2], Clinton O. Onyango[4], Benjamin McMahon[5], Kristan A. Schneider[2], Douglas Jay Perkins[2]

[1]Maseno University School of Medicine, Kisumu, Kenya, [2]University of New Mexico, Albuquerque, NM, United States, [3]Maseno University, Kisumu, Kenya, [4]University of New Mexico-Kenya Global Health Programs, Kisumu, Kenya, [5]Los Alamos National Laboratory, Los Alamos, NM, United States

Severe malarial anemia (SMA; Hb<6.0 g/dL) is a life-threatening complication of *Plasmodium falciparum* infection in children, characterized by ineffective erythropoiesis, systemic inflammation, and metabolic stress. Epidermal growth factor receptor (EGFR) signaling supports survival and repair mechanisms, and the ubiquitin-proteasome system (UPS) controls receptor ubiquitination, trafficking, and degradation. While transcriptional dysregulation has been studied, the contribution of the UPS post-translational mechanisms to host signaling responses in SMA remains poorly understood. We performed an integrative analysis of transcriptomic and proteomic data from children with SMA (n=16) and non-SMA (Hb ≥ 6.0 g/dL, n=19), presenting at a rural hospital in western Kenya. Peripheral blood total RNA was sequenced (Illumina® NovaSeq 6000), and clean reads were mapped to the human genome (GRCh38). Plasma proteins were profiled using the SomaScan Assay 7k v4.1, and relative fluorescence units were compared by generalized linear modeling. MetaCore™ network-building algorithm was used to infer functional interactions. A total of 847 genes and 224 proteins were differentially expressed (p<0.050) in children with SMA relative to non-SMA. The UPS-associated changes mapped onto the developing EGFR signaling pathway ($p$adj=8.208E-14 and $p$adj=1.302E-12) for transcriptomic and proteomic data, respectively. Canonical EGFR ligand/receptor interaction showed reduced EGF and EGFR at both mRNA and protein levels, while downstream signaling nodes showed opposing patterns (AKT1, MAPK1 mRNA↑/protein↓), indicating disrupted signal propagation. UPS-related changes were denoted by reduced UBE2D1, USP8, STAMBP, and RAB7A, and increased TSG101 and NEDD4L, consistent with altered ubiquitination and receptor sorting. Divergent patterns between the CBL protein and the CBLB transcript suggest compensatory responses within the E3 ligase network under post-translational control. Overall, EGFR signaling in SMA is reshaped by UPS-mediated mechanisms affecting receptor trafficking and downstream inflammatory and survival pathways.

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GUT MICROBIOME COMMUNITY STRUCTURE CORRELATES WITH PARASITE DENSITY AND ANTIMALARIAL EXPOSURE IN ZAMBIAN CHILDREN WITH *PLASMODIUM FALCIPARUM* MALARIA

Caitlin Bond[1], Abigail Glascock[2], Jean-Bertin Bukasa Kabuya[3], Jay Sikalima[4], Proscovia Banda[5], Charles Langelier[6], Ni Zhao[1], Ethan Gough[1], Jessica Queen[7], Matthew Michael Ippolito[7]

[1]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [2]Chan Zuckerberg Biohub, San Francisco, CA, United States, [3]Tropical Diseases Research Centre, Ndola, 8, Zambia, [4]Churches Health Association of Zambia, Ndola, Zambia, [5]Saint Paul's General Hospital, Nchelenge, Zambia, [6]University of California San Francisco, San Francisco, CA, United States, [7]Johns Hopkins University School of Medicine, Baltimore, MD, United States

Plasmodium falciparum malaria shows substantial clinical heterogeneity, ranging from asymptomatic infection to severe disease, with wide variation in parasite density and risk of recurrence. The biological drivers of this heterogeneity remain incompletely defined. Observational and preclinical studies suggest that gut microbial community structure may modulate infection and clinical risk and correlate with antimalarial drug exposure. To test our hypothesis that gut microbiome composition is associated with parasite density and antimalarial drug exposure in children with uncomplicated malaria , we collected stool samples from Zambian children under 5 years of age enrolled in a therapeutic efficacy study of artemether-lumefantrine and dihydroartemisinin-piperaquine. A total of 337 samples were collected from 159 children at enrollment, convalescence (1-2 weeks post-treatment), and recovery (5-9 weeks post-treatment), with parasitemia and recurrence assessed over 9 weeks of follow-up. Preliminary metagenomic and targeted metabolomic analyses were conducted on 12 baseline stool samples. Dirichlet multinomial mixture modeling identified two gut community state types (CSTs) in a 1:3 ratio: a *Bifidobacterium*-enriched CST and a CST with higher *Streptococcus* and Lachnospiraceae abundance. Baseline parasitemia was lower in the *Bifidobacterium*-enriched CST than in the other CST (median [IQR]: 10,000 [4,600–70,600] vs. 120,760 [75,040–136,000] parasites/μL; Wilcoxon p=0.03). The CSTs also showed distinct metabolic profiles, including differences in short-chain fatty acid production pathways. Pharmacokinetic analyses further suggested variation in lumefantrine and piperaquine exposure between CSTs. Together, these findings suggest that gut microbiome composition and function may be associated with parasite density and antimalarial drug exposure in children with uncomplicated malaria. Validation in the full cohort of 159 children will clarify these associations and advance understanding of microbiome-mediated mechanisms underlying malaria outcomes.

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YOUTH PERSPECTIVES ON GENE DRIVE TECHNOLOGIES FOR MALARIA CONTROL IN TANZANIA

Goodluck Zakaria Malika

Ifakara Heath Institute, Ifakara, United Republic of Tanzania

Gene drive-modified mosquitoes (GDMMs) present a promising innovation for malaria control in sub-Saharan Africa. However, their success depends on public understanding and acceptance, particularly among youth who represent a large share of the population. This study explored perceptions of youth (15-35 years) in Tanzania regarding malaria, the potential of GDMMs to accelerate control efforts, and the role youth could play in supporting such technologies. An exploratory qualitative study was conducted using nine focus group discussions with six youth groups, including motorcycle taxi riders, soccer teams, arts and crafts groups, sports coaches, and medical students. Participants were introduced to GDMMs through brief presentations followed by guided discussions. Transcripts were analyzed in NVivo 12 Plus using inductive and deductive thematic analysis. Youth expressed strong interest in contributing to malaria control and enthusiasm toward innovative tools such as GDMMs. However, understanding of malaria transmission, particularly the role of

mosquitoes, was limited, and malaria was often perceived as a routine and manageable illness. Participants expressed surprise at the national malaria burden and frustration over being uninformed and excluded from ongoing control efforts. Although some skepticism toward GDMMs emerged initially, youth indicated willingness to support malaria control initiatives if engaged early through trusted messengers, peer education, creative outreach, and sustained involvement. Findings highlight both the potential and unmet needs of youth in malaria-endemic settings. Meaningful, early, and inclusive engagement will be essential to ensure informed youth participation and build public support for emerging interventions such as GDMMs.

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COST AND IMPACT OF INTEGRATED SEASONAL MALARIA CHEMOPREVENTION AND INSECTICIDE-TREATED NETS CAMPAIGNS: EVIDENCE FROM JIGAWA STATE INTEGRATED CAMPAIGN, NIGERIA

Orezi Sophronia Adhekoyibo[1], Maureen Okoro[1], Emmanuel Obi[1], Sonachi Ezeiru[1], Rilwanu Mohammed[2], Nnenna Ogbulafor[3], Suzanne Van Hulle[4], Meredith Stakem[4]

[1]Catholic Relief Services, FCT, Nigeria, [2]Malaria Consortium, FCT, Nigeria, [3]National Malaria Elimination Programme, FCT, Nigeria, [4]Catholic Relief Services, Baltimore, MD, United States

Insecticide-Treated Nets (ITNs) and Seasonal Malaria Chemoprevention (SMC) are core malaria prevention interventions in Nigeria, yet they have historically been delivered through separate campaigns despite targeting overlapping populations. Evidence on the cost and impact of integration remains limited. In 2024, Jigawa State, Nigeria, implemented a full-scale integrated SMC/ITN campaign and we conducted a cost and coverage analysis. Budget data from the integrated campaign were compared with campaign budgets developed from historical expenditure data in comparable settings. Coverage outcomes including ITN distribution, household ITN ownership and use, and SMC Cycle 1 coverage were assessed using administrative records and post-campaign household surveys. Cost drivers and efficiency gains were analyzed across personnel, training, supervision, and meetings. The analysis showed that the integrated campaign was 28% less costly than estimated standalone campaigns, with approximately 75% of savings attributable to reduced personnel costs through shared household mobilization, combined supervision, and streamlined training. ITN distribution volumes were approximately 30% lower than previous campaigns, reflecting a strategic shift away from urban distribution, with slightly lower household ITN coverage (93% vs. 97-98%). However, ITN use among households was comparable to earlier campaigns (75% vs. 72-79%). SMC Cycle 1 coverage remained stable at approximately 96%, indicating no negative impact of integration. The cost per ITN distributed was the lowest recorded across recent campaigns, while the cost per net in use ($0.64) and cost per child reached with SMC ($1.52) were comparable to standalone campaigns. These findings support integrated campaigns as a viable strategy for malaria prevention in resource-constrained settings resulting in substantial cost savings, while sustaining coverage and cost-efficiency across both interventions.

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ADHERENCE BEYOND THE DISTRIBUTOR: FACTORS INFLUENCING CAREGIVER COMPLIANCE WITH DAY 2 AND 3 AMODIAQUINE DOSING IN SEASONAL MALARIA CHEMOPREVENTION (SMC) IMPLEMENTATION IN THE FEDERAL CAPITAL TERRITORY, NIGERIA

Daniel Chukwuyere Emeto, Olutomi Sodipo, Ezinwa Enyinnaya Uzukwu, Iheoma Nzekwe, Oluwafunmilayo Okpara, Keneth Maduka

Malaria Consortium, Abuja, Nigeria

Seasonal Malaria Chemoprevention (SMC) with sufadoxine-pyrimethamine plus amodiaquine (SPAQ) reduces malaria morbidity in children aged 3-59 months in areas of high seasonal transmission. While the Day 1 dose is directly observed by distributors, Days 2 and 3 rely on caregivers' administration. Poor adherence to unsupervised doses may reduce the protective effect of SMC and increase malaria risk. This study assessed caregiver adherence to Days 2 and 3 amodiaquine dosing and identified predictors of non-compliance in the Federal Capital Territory, Nigeria. Data from the Cycle 1 End-of-Cycle household survey were analyzed (1,231 caregiver-child pairs with documented Day 1 dose). Adherence was defined as verified administration of amodiaquine on Days 2 and 3 following a documented Day 1 dose. Descriptive statistics were used to estimate adherence rates, while bivariate and multivariate logistic regression models were used to identify predictors of non-compliance, including caregiver, programmatic, child, and contextual factors. Statistical significance was set at $p < 0.05$. Overall adherence to amodiaquine on Days 2 and 3 was 93%. Key predictors of non-compliance included lack of caregiver' trust in SMC efficacy (AOR 6.84; 95% CI: 2.04-22.95; p = 0.002), non-observance of directly observed treatment (DOT) by community drug distributors (CDDs) of the Day 1 dose (AOR 3.33; 95% CI: 1.60-6.95; p = 0.001), previous child adverse reactions to SPAQ (AOR = 5.89, 95% CI: 2.69-12.90, $p < 0.001$) and female caregivers were less likely to be non-compliant (AOR = 2.05, 95% CI: 1.04-4.04, p = 0.037). Caregivers' forgetfulness (35.5%) and child refusal (11.8%) were also reported as reasons for non-adherence. High administrative coverage may mask adherence to the full SPAQ course, which is essential for optimal protection against malaria. While adherence to the full course of SPAQ is high in the Federal Capital Territory, interventions should focus on building caregiver' trust and providing practical reminders to reduce forgetfulness. Strengthening these factors could further enhance the protective effect of SMC and reduce malaria risk among children.

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SEASONAL ROLLOUT OF R21 MALARIA VACCINATION AND CHEMO PREVENTION FOR CHILDREN IN KULE REFUGEE CAMP, ETHIOPIA, 2024

Y. G. Atnafu[1], A. Abbara[2], P. Keating[2], K. Siferaw[3], R. Wilson[2], D. Koang[4], K. Lavilla[1], B. Stringer[2], A. Tassawar[1], B. RAO[2], M. Ayalew[5], J. Loonen[6], C. Ariti[7], G. Mapiye[1], W. Mulanda[1], A. Pereira[8], A. Bestman[9], K. Velivela[9], B. Sahelie[1];

[1]MSF-HOLLAND, Addis Ababa, Ethiopia, Ethiopia, [2]MSF Manson Unit, London, United Kingdom, [3]Bahir-Dar University, Bahir-Dar, Ethiopia, [4]Gambella University, Gambella, Ethiopia, [5]MOH, Addis Ababa, Ethiopia, [6]MSF, Amsterdam, Netherlands, [7]LSHTM, London, United Kingdom, [8]MSF-HOLLAND, Amsterdam, Netherlands, [9]MSF-HOLLAND, Nairobi, Kenya

Malaria remains a major cause of morbidity among displaced populations living in hightransmission settings, where prevention efforts must be adapted to complex operational realities. This operational research evaluated the feasibility, acceptability, and programmatic performance of an integrated malaria prevention strategy combining the R21 malaria vaccine with agetargeted intermittent preventive treatment in children, implemented within existing malaria control activities in a refugee camp in Gambella Region, Ethiopia. An observational design was applied using routine surveillance data, vaccination and IPTc delivery records, and program monitoring information collected through primary health care services and community outreach platforms. The integrated delivery approach leveraged established service structures to optimize coverage and continuity of care. Implementation processes, service uptake, and operational indicators were assessed to examine feasibility and early programmatic outcomes. The combined introduction of R21 vaccination and IPTc was feasible within routine services and achieved high uptake among eligible children without disrupting ongoing health activities. Integration strengthened community engagement and improved coordination of malaria prevention interventions. Surveillance data demonstrated enhanced program responsiveness and timely detection of malaria trends following implementation. Health workers and caregivers reported high acceptability of the integrated approach, citing perceived effectiveness and convenience of combined service delivery. These findings suggest that integrating malaria vaccination with chemoprevention into existing malaria control strategies in humanitarian refugee settings is operationally feasible, acceptable, and capable of strengthening prevention efforts, supporting consideration for scaleup in similar highburden and emergency contexts.

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COMMUNITY-BASED DISTRIBUTION OF INSECTICIDE-TREATED NETS (ITNS). IN CONFLICT-AFFECTED NORTHERN MALI

Alassane Bangoura[1], Mady Cissoko[2], Aissata Kone[2], Cheikh Sidiya Gassama[1], Keita Fatoumata[1], Jeanbaptiste Kamate[1], Suzanne Van Hulle[3], Yameni Chrestien[4], Boubacar Sidiki Maiga[1]
[1]Catholic Relief Services (CRS), Bamako, Mali, [2]National Malaria Control Program - Mali, Bamako, Mali, [3]Catholic Relief Services, Baltimore, MD, United States, [4]Catholic Relief Services, Dakar, Senegal

Northern Mali faces a resurgence of multifaceted security conflicts, resulting in massive population displacement and limited access to healthcare services. Malaria remains the primary reason for medical consultations, accounting for 34% of cases in the general population. One of the key preventive measures against this scourge is sleeping under an Insecticide-Treated Net (ITN). Catholic Relief Services, in partnership with the National Malaria Control Program, organized an integrated ITN distribution campaign. To ensure its success, a strategy centered on community leaders, within a volatile security context, was developed. The approach involved a qualitative analysis of the security situation, which categorized districts into three risk levels: Level 1: Low risk. Level 2: Moderate risk. Level 3: High risk, characterized by a total lack of infrastructure and personnel. Humanitarian organizations were engaged to support transport and distribution via mobile teams to reach Internally Displaced and nomadic Populations (IDPs). The activity involved cascade training for 72 executives, 624 local supervisors, and 5,250 community leaders. These leaders were responsible for household registration and distribution, supported by members of Community Health Associations. Among 2,152,567 inhabitants, 98,362 were IDPs. Across these regions: 65% (15/23) of districts conducted household registration followed by data validation and subsequent distribution. 35% (8/23) of districts performed registration and distribution simultaneously. Overall coverage reached an impressive 99.64%. A dual payment mechanism for agents was developed: 60.4% of agents in areas with coverage were paid via mobile money, while 39.6% in uncovered areas were paid through the Regional Health Directorate. In insecure zones, distribution can no longer rely on traditional campaign models. The future lies in continuous distribution integrated into the standard package of health activities. To guarantee equitable access, it is crucial to strengthen the protection of health workers and adapt strategies dynamically based on the evolving security situation.

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BEYOND THE BED NET: CONTEXT-SPECIFIC ACCEPTABILITY OF MOSQUITO BITE PREVENTION TOOLS AMONG FOREST-EXPOSED COMMUNITIES IN CAMBODIA

Dyna Doum[1], David McIver[2], Neil Lobo[3], Allison Tatarsky[2]
[1]Health Forefront Organization, Phnom Penh, Cambodia, [2]UCSF Global Health Group, San Francisco, CA, United States, [3]Univ of Notre Dame, South Bend, IN, United States

Outdoor malaria transmission remains a significant challenge for high-risk populations in the Asia Pacific, necessitating the evaluation of tools that go beyond traditional bed nets. As part of Project BITE, this qualitative assessment utilized the COM-B model and Theoretical Domain Framework to evaluate the acceptability of tropical repellents, spatial repellents, and insecticide-treated clothing among forest-exposed communities in Kampong Speu and Mondulkiri, Cambodia. Between January and February 2023, we conducted 56 key informant interviews and 55 focus group discussions with 111 participants, including forest goers, dwellers, and rangers. Data were analyzed using Nvivo to identify behavioral barriers and facilitators. Results indicated high overall acceptability, though preference was context-dependent. Participants demonstrated strong psychological capability, identifying *Anopheles* mosquitoes as the primary vector and citing high financial and opportunity costs of illness as a motivator for protection. Tropical repellent was the most preferred tool due to its portability and ease of use during mobile forest activities. Conversely, spatial repellents were favored for enclosed domestic spaces but deemed impractical for the forest due to wind and rain. Treated clothing faced the lowest uptake, hindered by inconvenient treatment logistics and concerns regarding environmental contamination of water sources. Social opportunity was a primary facilitator, with Village Malaria Workers and wives acting as critical influencers for tool use. Despite reported side effects like skin irritation, 75% of participants expressed willingness to purchase a tropical repellent if available in markets. We conclude that prevention tools are highly acceptable when aligned with the mobility and environmental realities of end-users. Future scale-up should prioritize portable repellents and leverage social networks to sustain behaviors in the “last mile” of malaria elimination.

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THIRTY-THREE ROUNDS AND COUNTING: OPERATIONAL EVOLUTION OF AN URBAN INDOOR RESIDUAL SPRAYING PROGRAM ON BIOKO ISLAND, EQUATORIAL GUINEA, 2004 TO 2025

Lucas Ondo[1], David Galick[1], Liberato Motobe Vaz[1], Restituto Mba Nguema[1], Jeremias Nzamio Mba Eyono[1], Matilde Riloha Rivas[2], Wonder Philip Phiri[1], Carlos Alberto Guerra[3], Guillermo A. Garcia[4]
[1]MCD Global Health, Malabo, Equatorial Guinea, [2]National Malaria Control Program, Malabo, Equatorial Guinea, [3]MCD Global Health, Quito, Ecuador, [4]MCD Global Health, Hallowell, ME, United States

Global IRS coverage has fallen from protecting 151 million people in 2010 to 55 million in 2023 (WHO World Malaria Report 2024), driven largely by cost and operational complexity. Urban IRS programs, the hardest to execute and most resource-intensive, have been disproportionately affected. We describe the operational evolution of 33 IRS rounds implemented by the Bioko Island Malaria Elimination Project (BIMEP) on Bioko Island, Equatorial Guinea, between 2004 and 2025, documenting the adaptations made in response to specific program challenges and their relationship to coverage, productivity, and programmatic efficiency over time. The great majority of households on Bioko Island are in highly urban Malabo and surroundings, where household access, refusal rates, and dwelling structure present challenges not typical of rural IRS programs. Key adaptations were evidence-driven and iterative. These included a transition from two to one round per year as insecticide products evolved to provide longer residual protection, the adoption of a real-time spatial decision support system through the Campaign Information Management System enabling 100x100m sector-level planning and coverage monitoring, a shift in coverage target from 80% to 50% in urban areas following evidence from operational research, and the formalization of a gender-integrated spray workforce seconded by evidence of equivalent male and female operator productivity across multiple rounds. Insecticide rotation has remained part of the strategy, with VECTRON T500 currently in use. Over the program period, malaria prevalence in children aged 2 to 14 on Bioko declined from 42% in 2004 to 7.2% in 2025. Urban IRS sustained over decades and continuously refined through evidence is achievable and effective. BIMEP’s 33-round experience provides a replicable operational model at a moment when most programs have reduced investments in a tool that has proven effective against malaria.

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PATTERNS OF LONG-LASTING INSECTICIDAL NET COLLECTION AT FIXED DISTRIBUTION POINTS SUGGEST THE NEED TO REINFORCE MESSAGING TO IMPROVE USE OF THE SYSTEM

Liyu A. Teklemichael[1], Jordan Smith[1], Duncan McFarland[1], Honorio Borico Bielo[2], David Galick[2], Guillermo A. Garcia[1], Carlos A. Guerra[3]
[1]MCD Global Health, Hallowell, ME, United States, [2]MCD Global Health, Malabo, Equatorial Guinea, [3]MCD Global Health, Quito, Ecuador

In 2021, the Bioko Island Malaria Elimination Project shifted its long-lasting insecticidal net (LLIN) distribution strategy from triennial mass distribution campaigns (MDC) to fixed-distribution points (FDP) to minimize inefficiencies

and improve LLIN indicators. Initially, individual access and use, and use-to-access ratio improved for those who collected LLINs from fixed distribution points. The theory was that people who made the effort of approaching a distribution point would be more likely to keep and use their LLIN. Fixed distribution points were expanded in 2025 and the number of nets distributed has remained relatively constant each year, averaging 47 thousand nets annually. This number roughly matched the number of LLINs distributed in MDC every three years. However, LLIN indicators have recently dropped sharply. Here, we examined FDP data between 2021 to 2025 to assess the reasons behind this drop. Unique individuals accessing FDP were identified and classified as adequate users (those who revisited FDP every 1.5-3 years), over users (who revisited in <1.5 years), under users (who revisited in > 3 years), and single-visit users. Of 62,720 unique individuals using the system, 59,584 (90.5%) were single users and accounted for 78.5% of nets collected (n=181,884). Over-users comprised 5,342 individuals (8.1%) and 43,215 nets (18.7%). Adequate users comprised only 1.2% individuals (n=813) and accounted for only 5,688 nets (2.5%), while under-users (122; 0.2%) collected 779 nets (0.3%). While repeat collections by over-users accounted for a disproportionate fraction of nets collected, the findings suggest the main challenge for improving LLIN use and access remains getting single and under users to become adequate ones. Corrective measures for the system include reinforcing messaging on the importance of LLIN use to protect from malaria and the need to replace them at reasonable intervals (2-3 years) at FDP. This will be addressed through automated messages sent via AI-enabled chatbots triggered at FDP during collection. There is also a need to improve monitoring of over users to reduce inefficiencies of the system.

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MALARIA MINDS: BONNY ISLAND'S YOUTH SHIFT IN MALARIA PERSPECTIVES

Anastasia Isodje, Adetutu Adigwe, Uduak Iwatt
NLNG, Port Harcourt, Nigeria

Malaria continues to pose a significant public health burden in Nigeria, especially among children and adolescents. This research examined temporal shifts in malaria knowledge, attitudes, and preventive practices among secondary school students in Bonny, Rivers State, through comparative analysis of surveys conducted in 2023 (n=665) and 2026 (n=219). Using descriptive statistics, chi-square tests, and logistic regression, the study identified marked improvements in knowledge, as the percentage of students with good malaria knowledge increased from 20.9% in 2023 to 75.8% in 2026. Favourable attitudes towards prevention similarly increased from 42.1% to 90.4%, while preventive practices advanced more modestly, from 34.3% to 59.4%. Factors associated with knowledge included age, gender, religion, ward, class level, and survey year. Notably, senior students were approximately twice as likely to have good knowledge, and participants in 2026 were over 12 times more likely than those in 2023. These results suggest that improved knowledge fostered more positive attitudes, which—together with knowledge—promoted better preventive behaviours. Nonetheless, persistent challenges were noted, particularly inconsistent use of mosquito nets due to discomfort. The authors posit that although school-based malaria education proved efficacious, intensified efforts are required to surmount practical obstacles and translate knowledge gains into sustained practices.

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WHEN CONFIRMED CASES OUTPACE TREATMENT: APPLYING A CASE MANAGEMENT EFFICIENCY RATIO TO ROUTINE DHIS2 DATA TO QUANTIFY PAEDIATRIC MALARIA TREATMENT GAPS IN NORTH-WEST NIGERIA, 2022-2024

Michael Audu
Independent Researcher, Abuja, Nigeria

Ensuring every confirmed malaria case in a child under five receives artemisinin-based combination therapy is not a policy aspiration in north-west Nigeria, it is a daily operational challenge. Seasonal surges, procurement delays, and last-mile constraints routinely create a gap between diagnosed and treated cases, a gap rarely visible in annual programme summaries. We analysed DHIS2 malaria case management data from January 2022 to December 2024 across Kebbi, Sokoto, and Zamfara States using a Case Management Efficiency Ratio (CMER), defined as ACT treatments dispensed per confirmed uncomplicated case, disaggregated by age group and pregnancy status. Across the three states, 10,992,312 confirmed cases were recorded against 10,821,786 ACT treatments (CMER=0.98, gap=170,626). Children under five bore the largest burden in Sokoto:2,204,519 confirmed cases versus 2,074,789 treatments (CMER=0.94, gap=129,730). A single month accounted for most of this deficit. In May 2022, Sokoto recorded 199,599 confirmed under-five cases but dispensed only 47,924 ACTs (CMER=0.24), leaving 151,675 children untreated within thirty days. This aligns with a documented inter-quarter ACT stock depletion event and was not an isolated reporting anomaly. Zamfara showed a smaller gap (20,666 cases, CMER=0.98), while Kebbi showed a marginal surplus (CMER=1.01), suggesting redistribution or reporting timing effects. Routine DHIS2 data, analysed through treatment-aligned efficiency metrics, can identify when and where malaria case management fails, enabling timely, targeted action. Embedding CMER-based anomaly detection into NHLMIS and QAT cycles would transform routine reporting into an early-warning system for supply-chain disruptions.

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A QUANTITATIVE PRIORITIZATION FRAMEWORK FOR "SEEK AND DESTROY" LARVAL SOURCE MANAGEMENT: A DECISION SUPPORT MODEL FOR MALARIA ELIMINATION PLANNING IN UGANDA

Jesse Casanova
University of South Florida, Tampa, FL, United States

Malaria elimination in sub-Saharan Africa requires coordinated deployment of multiple interventions under resource constraints. Larval source management (LSM), particularly "Seek and Destroy" approaches targeting productive breeding habitats, is a high impact complement to standard vector control, yet Ministry of Health (MoH) planners lack systematic tools for comparing LSM against competing interventions in context specific settings. A multi-domain decision support model was developed for MoH program planners in Mbale Lukhonge District, Uganda. The model applies the formula Priority Score = Effect Size × Coverage Gap × Context Modifier across 79 controllable intervention variables spanning nine domains: LSM, insecticide-treated nets, indoor residual spraying, pharmaceuticals, case management, surveillance, health systems, behavior change communication, and housing. Effect sizes were sourced from peer-reviewed literature, and observational field studies. For variables without pooled effect size evidence. Thirty six uncontrollable contextual factors including conflict, climate, vector ecology, and land use inform a regional context modifier (Mbale baseline: 1.5). Under baseline conditions, top-priority interventions were larvicide application frequency, seasonal malaria chemoprevention coverage, habitat monitoring frequency, community health worker density, and IRS residual efficacy. Explicit evidence stratification allows decision-makers to distinguish high-confidence from assumption-based priority scores, strengthening the tool's utility for programmatic planning. The model is generalizable to other endemic settings through adjustment of the context modifier. This framework supports evidence informed integration of LSM into national elimination strategies and resource allocation under operational constraints.

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NDOLA HEALTH CENTRE MALARIA SURVEILLANCE AND RSHINY APP

Bertha Kasonde[1], Mike Chaponda[1], Sydney Mwanza[1], Taonga Banda[1], Amy Wesolowski[2], William Moss[3]

[1]National Health Research and Training Institute, Ndola, Zambia, [2]John Hopkins School of Public Health, Baltimore, MD, United States, [3]John Hopkins School of Public Health, Ndola, MD, United States

Despite sustained investment in malaria control and elimination throughout Zambia, reported cases continue to rise in Ndola District. Health facility surveillance is a critical component of public health management, enabling the continuous collection, analysis, and interpretation of public health data. A health facility surveillance system was set up to characterize spatial and seasonal malaria transmission dynamics in peri-urban and urban Ndola. Digital Surveillance involved weekly reporting of malaria case counts and RDT positivity via SMS, and Dried Blood spot (DBS) surveillance. The R Shiny app was fed by the weekly SMS -RDT confirmed cases (age-stratified). 2,790 participants were enrolled, with the majority being males (54.9%). The mean age of the participants was 23, with the median 21. To understand transmission dynamics, a three-fold analytical framework was used, namely granular Surveillance, zone-level visualization of malaria data for targeted response, including Spatial Heterogeneity, focusing on peri-urban vs urban patterns and local vs travel-associated cases, and Seasonal Dynamics Transmission. Urban Hotspots have the highest total number of malaria cases, largely due to the high population density. Demographic impact shows a significant disease burden, specifically among adults aged 15 and older. The R-Shiny application was developed to identify malaria hotspots, allowing swift, data-informed decisions for disease control to be made. Weekly TPR showed clear seasonal fluctuations in both peri-urban and urban settings (SAI 2.22 vs 1.58). Peri-urban facilities revealed a higher overall transmission intensity, whilst Urban facilities exhibited stronger seasonal amplification. Both settings demonstrated rainfall-associated seasonality. There was masked facility-level heterogeneity observed, with persistent peri-urban hotspots and sub-health centre clustering masked in facility aggregates. 98.3% of confirmed cases reported no travel; however, there was predominantly local transmission & limited international importation.

6298

USING LOT QUALITY ASSURANCE SAMPLING TO DETECT MICRO-LEVEL GAPS IN SEASONAL MALARIA CHEMOPREVENTION DELIVERY IN OYO STATE, SOUTHWESTERN NIGERIA

Taiwo Iyabode Olarinde[1], Ayodeji J. Balogun[1], Moses E. John[1], Jennifer Ugochi Chukwumerije[2], Catherine O. Onoriede[1], Olabisi Adebo Ogunmola[3], Bronwen Davies[4], Chibuzo Oguoma[5], Rilwan Jega[6]

[1]Malaria Consortium, Ogbomoso, Nigeria, [2]Malaria Consortium, Abuja, FC, Nigeria, [3]Malaria Consortium, Maitama Abuja, Nigeria, [4]Malaria Consortium, London, United Kingdom, [5]Malaria Consortium, Abuja, Nigeria, [6]Malaria Consortium, Sokoto, Nigeria

Seasonal Malaria Chemoprevention (SMC) in Nigeria routinely achieves high aggregate coverage. However, aggregate indicators can mask micro-level operational gaps that compromise implementation quality. Lot Quality Assurance Sampling (LQAS) provides a structured, surveillance-oriented, decision-making approach for classifying subdistrict performance using predefined thresholds within routine monitoring systems. This study assessed the use of LQAS to detect micro-level gaps in SMC delivery and inform targeted corrective action in Oyo State, Nigeria. A cross-sectional operational research study was conducted using End-of-Cycle survey data from four SMC cycles across selected Local Government Areas in Oyo State. Supervision areas were defined as LQAS "lots," with 25 caregiver–child pairs assessed per lot per cycle. Performance thresholds were set at 100% for household visitation and SMC coverage, and ≥95% for direct observation of Day 1 sulfadoxine–pyrimethamine plus amodiaquine (SPAQ) administration and completion of Day 2 and Day 3 doses. Decision values (d) were 23 for 100% targets and 22 for 95% targets; lots exceeding (n – d) failures were classified as requiring corrective action. LQAS classifications compared with aggregate coverage revealed subpopulation gaps obscured in routine surveillance. Targeted supportive supervision was implemented in failed lots, and changes in lot status across cycles were assessed descriptively. Despite aggregate state-level SMC coverage exceeding 95%, LQAS identified 12–18% of lots per cycle as failing at least one indicator. Direct observation of Day 1 SPAQ administration was most frequently failed. Following targeted supervision, over 70% of previously failed lots met acceptable thresholds by Cycle 3. LQAS effectively identified micro-level gaps obscured by aggregate indicators. Integrating LQAS into routine SMC surveillance strengthens implementation quality and enhances programme performance. Keywords: Seasonal Malaria Chemoprevention; Lot Quality Assurance Sampling; Operational Research; Program Monitoring and Evaluation; Malaria Control

6299

ECONOMIC BURDEN OF ACUTE UNCOMPLICATED MALARIA IN SUBSAHARAN AFRICA AND COST PARAMETERS FOR PREVENTION AND TREATMENT: A SYSTEMATIC LITERATURE REVIEW (SLR)

Yvonne Geissbühler[1], Jean-Bernard Gruenberger[1], Namita Kaushik[2], Thej Kumar Nallagangula[2], Justice Nonvignon[3], Richmond Owusu[4]

[1]Novartis Pharma AG, Basel, Switzerland, [2]Novartis Healthcare Private Limited, Hyderabad, India, [3]Management Sciences for Health, Arlington, VA, United States, [4]School of Public Health, University of Ghana, Accra, Ghana

Acute uncomplicated malaria (AUM) accounts for ~95% of all malaria cases and is a major public health and economic challenge in sub-Saharan Africa (SSA). Evidence on current economic burden of AUM and malaria prevention costs is fragmented. To support policy-relevant impact and cost-effectiveness modelling, we conducted an SLR following PRISMA guidelines (CRD420251013846). Two independent reviewers screened and extracted data across household, provider, and societal perspectives. A total of 114 studies from 130 publications (1st Jan 2014 - 6th March 2026) were included from 3,825 identified records. We converted cost data from local currencies into 2024 US dollars (US$) to ensure consistency in comparisons. Across 51 AUM studies, total costs per episode ranged from US$1.78 - 40.90. Direct medical costs were US$0.27 - 35.63, while household out of pocket payments were US$0.80 - 29.17. Productivity losses (US$5.63 - 19.60) often accounted for over 50% of the total household burden. Cost patterns were similar across rural versus urban settings (US$2.90 - 36.90 versus US$3.80 - 35.30). Cost heterogeneity was driven by variation in study perspective, health system structure, and public versus private care. The vector control and prevention costs for malaria (63 studies) also varied widely by country, intervention type, and program components. Long-lasting insecticidal net distribution costs ranged from US$0.76 - 6.61 per net, indoor residual spraying costs varied from US$0.73 - 7.76 per person targeted and chemoprevention costs were US$0.41 - 1.78 per monthly course. Integrating these parameters highlights economic tradeoffs between prevention and case management. The AUM imposes a substantial and variable economic burden across SSA, largely driven by productivity losses and household direct costs. This up-to-date SLR provides consolidated evidence on prevention and treatment costs, enabling robust economic evaluation of emerging antimalarial therapies. The findings offer standardized cost parameters to support evidence-based decision-making for malaria control, elimination, and resistance management strategies.

6300

MALARIA DATA QUALITY PRACTICES AND BARRIERS USING MALARIA COMMODITY LOGISTIC SYSTEM STANDARD OPERATING PROCEDURE AMONG HEALTHCARE PROVIDERS IN OYO AND NASARAWA STATES, NIGERIA

Oludayo Osulolale Adekunle[1], Ademola Adelekan[2], Job Ozoga[3], Oyewole Lawal[4]

[1]GHSC-PSM Nigeria, ABUJA, Nigeria, [2]McPherson University, Seriki Sotayo, Nigeria, [3]Nasarawa State Primary Health Care Development Agency, Lafia, Nigeria, [4]Oyo State Ministry of Health, Ibadan, Nigeria

Malaria remains a major public health burden in Nigeria, which accounts for a significant proportion of global malaria morbidity and mortality. Reliable malaria logistics data are critical for effective commodity management; however, persistent concerns exist regarding data quality at service delivery points. This study assessed malaria data quality practices and identified barriers as informed from the use of Standard Operating Procedures (SOPs) in managing Malaria Commodity Logistics System/ Malaria Products Logistics System (MCLS/MPLS) among healthcare providers in Oyo and Nasarawa States, Nigeria. A descriptive mixed-methods design was employed. Quantitative data were collected from 300 malaria focal persons across selected health facilities using a six-stage sampling technique, while qualitative data were obtained through 22 key informant interviews with local government and state-level malaria program stakeholders. Findings revealed that only 13.0% of respondents demonstrated good malaria data quality practices, with higher performance in Oyo State (19.4%) compared to Nasarawa State (9.0%). Individual-level barriers constituted the largest proportion (39.7%), followed by environmental barriers (29.3%) and organizational barriers (11.3%). Key individual barriers included the multiplicity of SOPs (52.2%) and inadequate skills (27.2%). Organizational challenges included weak adherence promotion (15.1%) and limited training and resources (13.9%), while environmental constraints were dominated by work-life balance (33.3%) and family obligations (27.2%). The study highlights substantial gaps in malaria data quality practices, largely driven by behavioral and systemic factors rather than technical deficiencies. Strengthening supportive supervision, improving access to SOPs, enhancing continuous training, and addressing workforce and motivational challenges are critical to improving data quality, thereby optimizing malaria commodity management and advancing malaria elimination efforts in Nigeria.

6301

QUALITY ASSESSMENT OF MALARIA INCIDENCE DATA REPORTED THROUGH THE HEALTH MANAGEMENT INFORMATION SYSTEM BASED ON DIRECT COMPILATION FROM OUTPATIENT REGISTERS USING A SYSTEMATIC QUALITY CONTROL PROCESS

Azuka Iwegbu[1], Abiola Oluwaseun Oluwagbemiga[1], Adeola Temidayo[2], Folayan Waheed[2], Tarekegn A. Abeku[3]

[1]Malaria Consortium Nigeria, Akure, Nigeria, [2]Ondo state ministry of health, Akure South, Nigeria, [3]Malaria Consortium, London, United Kingdom

Reliable and timely malaria data are fundamental for guiding decision-making for effective implementation and evaluation of interventions. A study is ongoing to assess impacts of insecticide-treated net campaigns in Ondo State. One component involved compiling malaria data from outpatient registers in sentinel health facilities using a paper-based tool to monitor incidence trends. In this analysis, digitised recompiled data generated through a systematic quality control process were compared with monthly malaria data reported through the routine Health Management Information System (HMIS) to assess reliability of routinely reported data. Malaria data from 31 health facilities in Ondo State for 2023 were used for comparison. Recompilation of outpatient register data was carried out by health facility staff under structured supervision, which included periodic visits from local government authorities and the State Ministry of Health. Two independent data clerks entered the recompiled data into EpiData, and a supervisor conducted validation to ensure accuracy. Discrepancies between the recompiled data and monthly malaria figures reported through HMIS forms and entered into the DHIS2 platform were assessed using Stata.Monthly confirmed malaria cases were over-reported in the HMIS by an average of 8%. Among the 31 health facilities, 4 facilities (13%) reported monthly confirmed cases that consistently matched the recompiled data and were therefore considered fully accurate. Another 9 facilities (29%) reported matching figures for at least 9 of the 12 months. In contrast, 3 facilities (10%) had monthly reports that did not align with the recompiled data in any month. In 2023, HMIS totals for confirmed cases were within 5% of the recompiled figures for 52% of facilities, whereas discrepancies greater than 20% were observed in 16% of the facilities.The study showed moderate discrepancies between malaria data compiled. However, robust quality control system is required to improve the reliability of data reported through the HMIS system for evidence-based decision-making in planning, implementation and evaluation of interventions.

6302

DISTRICT-LEVEL READINESS FOR MALARIA ELIMINATION RESPONSE IN ZANZIBAR: RESULTS FROM A CAPACITY ASSESSMENT TO INFORM DECENTRALIZED PLANNING

Richard Silumbe[1], Saidi Mgata[1], Bakar Shariff Fakih[2], Joseph Joachim[3], Katelyn Woolheater[4], Maggie Afenu[5], Christian Lengeler[6], Paul Bouanchaud[7], Christopher Lourenco[8]

[1]PSI Tanzania, Dar es Salaam, United Republic of Tanzania, [2]Swiss Tropical and Public Health Institute, Allschwil, Switzerland, [3]Swiss Tropical Public Health Insititue, Basel, Switzerland, [4]Austin, TX, United States, [5]PSI, Lawrence, MA, United States, [6]Swiss Tropical & Public Health Institute, Allschwil, Switzerland, [7]PSI, Washington, DC, United States, [8]PSI, Hopedale, MA, United States

Zanzibar has made substantial progress toward malaria elimination, with surveillance and case investigation systems capable of detecting most malaria infections. As transmission declines, rapid investigation and response to individual cases becomes increasingly important. Decentralizing operational responsibilities to district health teams may improve the speed and effectiveness of malaria response, but information on district-level readiness for elimination activities remains limited. A comprehensive district capacity assessment was conducted across Zanzibar to evaluate readiness for decentralized malaria elimination activities. The assessment examined key domains including surveillance capacity, human resources, transportation and logistics, data use, and coordination mechanisms. Data were collected through structured interviews, document review, and facility observations. Results indicate substantial variation in readiness across districts. While most districts demonstrated strong experience with malaria surveillance activities, gaps were identified in operational capacity, including transportation availability for case investigation, dedicated staff time for elimination activities, and integration of surveillance data into routine decision-making. Coordination between district health teams and national malaria program structures also varied, highlighting opportunities to strengthen operational linkages. Findings from the assessment are informing the development of tailored district decentralization plans designed to strengthen operational capacity while maintaining national oversight. These results highlight the importance of assessing local system readiness when transitioning toward decentralized malaria elimination strategies.

6303

SPATIAL RESAMPLING OBSCURES TEMPORAL CHANGE IN MALARIA PREVALENCE: EVIDENCE FROM SIMULATED MALARIA INDICATOR SURVEYS

Richard Graciano Kamwezi

Malawi Liverpool Wellcome Programme (MLW), Blantyre, Malawi

Reliable measurement of temporal changes in malaria prevalence is essential for evalating control programs. However, spatial heterogeneity and the resampling of srvey locations can introduce substantial uncertainty. This study quantified the impact of enumeration area (EA) resampling on the precision of prevalence trends using modeled *Plasmodium falciparum*

maps rom 2010 to 2017. We simulated 100 Malaria Indicator Survey (MIS) EA draws to compare a standard resampled design, where EAs were selected independently each year, against a fixed design that retained the same EAs across years. Simulations revealed substantial variability in prevalence estimated arising solely from random spatial resampling particluarly at the regional level where the northern region of Malawi showe a range of simulated values up to 12.9 percentage points. While the central tendency of prevalence estimates remained consistent and unbiased between both sampling designs, the fixed design significantly reduced the variance of estimated changes between surveys. In contrast, the resampled design amplified variability, suggesting that changing survey locations introduces spatial noise that can obsecure true temporal trends and potentially mislead programmatic evaluations. These findings indicate that random resampling of EAs between MIS rounds reduces reliability of inferred trends. Incorporating fixed or partially fixed EAs across survey years could significantly improve precision of prevalence estimates and strengthen programmatic evaluation, especially in increasingly heterogenous low-transmission settings where sistinguishing genuine epidemiological change from sampling artifacts is critical.

6304

PERSPECTIVE OF THE STAKEHOLDERS ON AND THEIR ENGAGEMENT IN MALARIA MOLECULAR SURVEILLANCE IN MAINLAND TANZANIA

Doris David Mbata[1], Daniel Protasy Challe[2], Dativa Pereus[3], Daniel Anderson Petro[4], Misago D. Seth[1], Catherine Bakari Mvaa[5], Rashid Ally Madebe[1], Salehe Saidi Mandai[1], Angelina J. Kisambale[1], Hussein J. Semboja[5], Gervas A. Chacha[1], Happiness A. Mwailunga[5], Laurencia S. Tangawiz[5], Gideon M. Isaya[5], Baraka W. Mbuba[5], Ninja S. Bisaga[5], Edwin Liheluka[1], Sijenunu Aaron[6], Daniel S. Mbwambo[6], Abdallah Lusasi[6], Samwel Lazaro[6], Stella Kajange[7], Solomon Mwakasungula[5], Celine Isaack Mandara[1], Vedastus W. Makene[8], Deusdedith R.S. Ishengoma[5]

[1]National Institute for Medical Research, Dar Es Salaam, United Republic of Tanzania, [2]National Institute for Medical Research, Tanga, United Republic of Tanzania, [3]Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania, Dar Es Salaam, United Republic of Tanzania, [4]University of Dar es Salaam, Dar Es Salaam, United Republic of Tanzania, [5]Ifakara Health Institute, Dar Es Salaam, United Republic of Tanzania, [6]National Malaria Control Programe, Dar Es Salaam, United Republic of Tanzania, [7]Prime Minister's Office, Regional Administration and Local Government, Dodoma, Tanzania, Dodoma, United Republic of Tanzania, [8]Open University of Tanzania, Dar es Salaam, United Republic of Tanzania

As global malaria efforts shift from control to elimination, traditional surveillance approaches such as microscopy and rapid diagnostic tests for detecting malaria parasites are increasingly supplemented by malaria molecular surveillance (MMS) based on application of genomic and bioinformatics tools. However, successful integration of MMS into routine health malaria surveillance requires meaningful stakeholders' engagement beyond research implementation. True ownership of research findings occurs when stakeholders are engaged from the identification of the research idea, implementation of research to sharing and use of evidence for policy and decision making. This cross-sectional qualitative study explored stakeholders' perspectives on and their engagement in MMS, with a focus on data processing (notably packaging, presentation, sharing of findings) and utilization of evidence generated by MMS for policy and decision making in Mainland Tanzania. Key informant interviews (KIIs) were conducted with 32 stakeholders (purposefully selected at the district, regional, and national levels) and used to capture the experience and perspectives of different stakeholders and their involvement in MMS. Interviews were audio-recorded, transcribed verbatim, and analyzed using thematic framework analysis. The findings revealed that stakeholder engagement in MMS is heterogeneous across health system levels. At the district and regional levels, engagement is primarily operational, with stakeholders focusing on data generation, field implementation, and routine surveillance. In contrast, national-level stakeholders are more engaged in data interpretation, evidence synthesis, and translation of findings into policy, guidelines, and strategic interventions. This disconnect highlights gaps in feedback loops and limited involvement of subnational stakeholders in higher-level decision-making processes. Strengthening inclusive stakeholder engagement frameworks will be critical to enhance ownership, improve data utilization, and ensure sustainability of MMS in malaria-endemic settings

6305

TRIPLESLIDE: AN IMPROVED, REGRESSION-BASED FRAMEWORK FOR DETERMINING ANTIGEN COMPOSITION IN PROTEIN-PROTEIN CONJUGATE VACCINES AGAINST MALARIA

Christopher G. Rowe, Ana M. Ortega-Villa, Beth B. Chen, Karine N. Reiter, Raul Herrera, Fernando Cabezas Mejia, Puthupparampil V. Scaria, Michael Proschan, David L. Narum, Patrick E. Duffy

National Institutes of Health/NIAID, Bethesda, MD, United States

Our malaria vaccine program has advanced protein-protein conjugates from preclinical testing through early-stage clinical trials toward potential commercial licensure. Determining antigen-specific protein concentration is critical for product release. To address this, we developed an expanded statistical framework for estimating antigen composition from amino acid analysis (AAA) data. TripleSlide is a Shiny application that integrates regression modeling, diagnostics, variance estimation, and automated reporting in a web-accessible platform with printable outputs and reviewable code. Antigen composition is commonly determined using linear least-squares regression (LSR) applied to AAA data, in which residue compositions from a subset of acid-stable amino acids in each unconjugated protein serve as independent variables and conjugate AAA measurements serve as the dependent variable in an Excel-based regression model. We expanded this approach to include all detectable amino acid residues rather than a limited subset. The method incorporates assay diagnostics, graphical evaluation of model fit, assessment of inter-replicate variability, and statistical outlier and influence analyses to identify residues that disproportionately affect model suitability or estimation precision. Both Delta and bootstrap variance estimation methods were implemented to better account for potential collinearity between antigen and carrier proteins. Over 100 conjugates were analyzed using the expanded method, producing % (w/w) antigen estimates comparable to prior methods, often with narrower confidence intervals. Improved variance estimation enhanced characterization of uncertainty, particularly in the presence of collinearity, while integrated diagnostics facilitated evaluation of assay performance and the impact of residue exclusion on final estimates. We are using TripleSlide for dose determination in malaria vaccines, including Pfs230D1-CRM197, our lead transmission-blocking candidate currently in Phase II trials.

6306

MALARIA TRANSMISSION-BLOCKING NANOPARTICLE VACCINES ELICIT POTENT RESPONSES UPON VACCINATION INDIVIDUALLY AND IN COMBINATION

Nichole Diane Salinas[1], Rui Ma[2], Thayne Henderson Dickey[3], Myesha Singleton[1], Tarik Ouahes[1], Nida Delbe[4], Holly McAleese[4], Luwen Zhou[5], Carole A. Long[6], Kazutoyo Miura[7], Lynn Lambert[4], Niraj H. Tolia[2]

[1]NIAID, Silver Spring, MD, United States, [2]National Institutes of Health, Bethesda, MD, United States, [3]NIH/NIAID/LMIV/HPISV, Bethesda, MD, United States, [4]NIAID, Bethesda, MD, United States, [5]NIH, Rockville, MD, United States, [6]National Institutes of Health, Rockville, MD, United States, [7]VRC/NIAID/NIH, Bethesda, MD, United States

There is an alarming trend of increased numbers of cases of and deaths caused by malaria parasites since the 2020 COVID-19 pandemic, a reversal of trends prior to 2020 (World Malaria Report 2025). Tragically, the majority of these deaths were children under the age of 5. Coupled with the rise in parasite resistance to antimalaria drugs, a malaria vaccine is desperately needed. One category of potential malaria vaccines is transmission blocking vaccines (TBVs). TBVs function by breaking the continuous cycle of infection between the human host and the mosquito vector, specifically by

reducing the mosquito infection and blocking the subsequent reinfection of another human host. A number of gametocyte proteins have been identified as TBV candidates including Pfs230, Pfs48/45, and Pfs25 all of which have transmission reducing activity (TRA) associated with them. TRA has been mapped to domain 1 of Pfs230, domain 3 of Pfs48/45, and multiple regions of Pfs25. Although TRA has been associated with all three proteins, monomeric proteins do not produce potent TRA upon vaccination. Here, we show that single-component self-assembling nanoparticles composed of the fusion of these monomeric proteins individually to ferritin results in vaccines that have high stability, homogeneity, and production yields. These nanoparticle vaccines also elicit potent antibody responses with high TRA after vaccination when formulated in two distinct adjuvants suitable for translation to human use (Alhydrogel and AddaS03). Potent antibody responses and high TRA are also seen when the individual nanoparticles are combined in a single formulation. These nanoparticle vaccines may play a key role in malaria control and have the potential to improve production pipelines and the cost of manufacturing of a potent and durable TBV.

6307

METHODOLOGY FOR ASSESSING FUNCTIONAL ACTIVITY OF *PLASMODIUM VIVAX* TRANSMISSION BLOCKING VACCINES

Heather A. Goodman, Jen CC Hume, Holly M. Torano, Joel Goldberg, Shanan Immel, John Woodford, Julia Zemskova, Emily R. Higbee, Patrick E. Duffy

NIH/NIAID, Bethesda, MD, United States

Plasmodium vivax (Pv) controlled human malaria infection (CHMI) studies have recently advanced utilizing the induced blood stage model of infection where cryopreserved asexual parasites are inoculated intravenously to initiate PvCHMI in study participants. Development of parasitemia and gametocytemia is monitored closely by qPCR and blood from 'donor' participants can be combined with sera from Pv transmission blocking vaccine (TBV) trial volunteers and fed to *Anopheles* mosquitoes to test functional activity. This method for testing transmission blocking and transmission reducing activity of potential Pv TBVs is analogous to the well-established, standard membrane feeding assay utilized for evaluation of *P. falciparum* vaccine candidates. Here we present the methodology used for evaluating a PvTBV candidate in a non-endemic malaria setting utilizing donor gametocytes from PvCHMI donors. We discuss assay design, feeding parameters, assay controls, effect of variation in parasite and gametocyte levels between PvCHMI donors, and management of potential blood group incompatibility between donor blood and test sera. Lastly we provide data for testing functional activity using gametocyte concentrated Pv donor samples to help determine the optimal assay design for reproducible results.

6308

STRUCTURE-BASED DESIGN OF *PLASMODIUM FALCIPARUM* EBA-175 F2 DOMAIN

Ajay Kumar[1], Richi Gupta[1], Thayne Henderson Dickey[1], Ababacar Diouf[2], Nichole Diane Salinas[1], Myesha Singleton[3], Sachy Orr Gonzalez[3], Tarik Ouahes[3], Carole A. Long[3], Lynn E. Lambert[3], Niraj H. Tolia[1]

[1]Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States, [2]Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States, [3]Vaccine Development Unit, Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States

Erythrocyte Binding Antigen 175 (EBA-175) is a potential malaria vaccine candidate, and a merozoite ligand that binds to the receptor glycophorin A (GpA) on erythrocytes facilitating parasite invasion. Region II (RII) of EBA-175 contains the F1 and F2 subdomains that are critical for GpA binding and comprises the receptor-binding domains. F1 and F2 are Duffy-binding like domains structurally similar to the *Plasmodium vivax* Duffy Binding Protein. Naturally acquired antibodies targeting F2 prevent erythrocyte invasion and are associated with protection from disease, however strain-specific responses have been observed. The mouse monoclonal antibodies R217 and R218 recognize F2 and F1, respectively; however, only R217 neutralizes parasite growth. In this study, we employed a structure-based design approach in an attempt to create stabilized F2 immunogens that may overcome strain-specific responses potentially by focusing the immune response to potently neutralizing antibody epitopes. We utilized *in vitro* screening methods to identify lead immunogens with improved affinity and specificity for the antibody R217 and higher production yields. Preclinical evaluation in animals by immunization of rats with these designed immunogens resulting in high titers of antibodies, however, strain-transcending responses were not observed. Collectively, these findings indicate that computational design generates immunogens with improved affinity and production yield, but these designs are incapable of overcoming the multiple invasion pathways and strain-specific response unique to EBA-175. It is plausible that a combination of these immunogens with other targets may be a viable path for malaria vaccine development.

6309

COMPUTATIONAL DESIGN OF THE FUNCTIONALLY CRITICAL F1 DOMAIN OF EBA-140

Shashank Shekhar[1], Thayne Henderson Dickey[1], Nichole Diane Salinas[1], Myesha Singleton[2], Tarik Ouahes[2], Sachy Orr Gonzalez[2], Ababacar Diouf[3], Carole A. Long[3], Lynn E. Lambert[2], Niraj H. Tolia[1]

[1]Host-Pathogen Interactions and Structural Vaccinology Section, Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States, [2]Vaccine Development Unit, Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States, [3]Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States

Plasmodium falciparum EBA-140 is a potential blood-stage vaccine candidate that is involved in reorientation and tight junction formation during red blood cell invasion. Region-II (RII) of EBA-140 contains F1 and F2 Duffy-binding like (DBL) domains that engage glycophorin C on host RBCs in a sialic acid-dependent manner. Immunization with F1 elicited a greater growth inhibitory immune response than immunization with RII, as antibodies raised against F2 are mostly non-neutralizing. However, none of the monoclonal antibodies characterized accessed the sialic acid binding site within F1, suggesting this is a subdominant epitope. Here, we leveraged the established computational design platform in an attempt to improve the efficacy of EBA-140 by driving the immune response toward the F1 domain. F1 immunogens with amino acid changes outside the sialic acid binding site had improved production characteristic over the wildtype F1. Key designs with optimal biophysical characteristics were back mutated to restore any potentially unidentified neutralizing epitopes. The final designs showing high production profiles and high percent identities to the wildtype F1 were further evaluated by vaccination in preclinical animal studies, however strain-transcending growth inhibitory activity was not observed. The results indicate that despite improved biophysical properties, computational design cannot overcome the multiple pathways and strain-specific responses unique to EBA-140. Further development of these F1 focused designs in combination with other antigens is plausible and may result in strain-transcending responses desirable for a malaria vaccine.

6310

INITIAL EVALUATION OF *PLASMODIUM VIVAX 230-KDA* GAMETE SURFACE PROTEIN DOMAIN 1 (PVS230D1)

Daming Zhu, Weili Dai, Holly McClellan, Alec Allee-Munoz, Karine Reiter, Nicholas J. MacDonald, Kelly M. Rausch, David L. Narum, Patrick Emmet Duffy

Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Disease, National Institutes of Health, Bethesda, MD, United States

Plasmodium vivax P230 domain 1 (Pvs230D1) is a promising malaria transmission-blocking vaccine (TBV) candidate. Pvs230 is expressed by gametocytes in the human host and displayed on the surface of gametes in the mosquito midgut following ingestion of an infected blood meal. Antibodies directed against Pvs230 can disrupt parasite development in the mosquito and thereby block malaria transmission. To enhance the immunogenicity of recombinant Pvs230D1 expressed in *Pichia pastoris*, the antigen was conjugated to the recombinant, non-toxic *Pseudomonas aeruginosa* ExoProtein A (rEPA) carrier protein to generate a conjugate TBV candidate. As an active component of a conjugated malaria TBV, extensive characterization of Pvs230D1 is required by regulatory agencies prior to its use in human clinical trials. In this study, Pvs230D1 was manufactured in compliance with current good manufacturing practices (cGMP). Initial evaluation of the cGMP lot included tests for appearance, endotoxin by Limulus amebocyte lysate (LAL) assay, sterility, general safety, rabbit pyrogenicity, residual host cell DNA by quantitative PCR (qPCR), carbohydrate content by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD), residual host cell proteins (HCP) by slot blot analysis, and demonstration of HCP removal during purification by Western blot. Additional characterization included protein content by UV absorbance at 280 nm (A280) and bicinchoninic acid (BCA) assay, identity by amino acid composition analysis, pH, SDS-PAGE with Coomassie blue staining, capillary gel electrophoresis (CGE), N-terminal sequencing, electrospray ionization mass spectrometry, and Western blot analysis. Protein integrity and purity were further assessed by SDS-PAGE, CGE, reverse-phase ultra-performance liquid chromatography (RP-UPLC), and size-exclusion ultra-performance liquid chromatography (SEC-UPLC). Results demonstrated that the cGMP-manufactured Pvs230D1 met all established release specifications and is suitable for use as an active protein component in malaria TBV formulations for human clinical trials.

6311

CIRCULAR RNA VACCINES AGAINST MULTIPLE LIFE STAGES OF *PLAMODIUM FALCIPARUM*

Nicholas C. Solek[1], Katherine Prieto[2], Giulia Costa[3], Amrita Dhindsa[2], Danton Ivanochko[2], Matthijs Jore[4], Elena Levashina[3], Jean-Philippe Julien[2], Bowen Li[1]

[1]University of Toronto, Toronto, ON, Canada, [2]The Hospital for Sick Children, Toronto, ON, Canada, [3]Max Planck Institute for Infection Biology, Berlin, Germany, [4]Radboud University Medical Center, Nijmegen, GE, Netherlands

Plasmodium falciparum is the primary causative agent for malaria, responsible for the deaths of roughly 600,000 people in 2024. *P. falciparum* undergoes several morphological changes throughout its life cycle, progressing through infectious, blood, and transmissive stages. As of 2023, two vaccines have been deployed targeting only the infectious stage of the malarial parasite. Despite this, infection rates have not decreased, pointing to a need for improved preventative measures. Ribonucleic acid (RNA) -lipid nanoparticle (-LNP) vaccines have demonstrated efficacy and modularity for defense against infectious diseases. The RNA platform enables facile manufacturing and co-delivery of multiple immunogens at once, establishing it as a premier technology for use in multi-stage vaccines. However, first-generation RNA vaccines suffer from limited potency and durability of immune responses, owing to transient immunogen expression and poorly targeted delivery systems. Circular RNA (circRNA) and immune cell-optimized LNPs address these challenges. CircRNA shows enhanced stability of protein expression, bolstering immune responses. Optimizing LNPs for transfection of immune cells further improves adaptive immune responses. Here, we developed circRNA-LNPs for use in multi-stage malaria vaccines. We delivered multi-stage circRNA-LNP vaccines in vivo, individually and in multiplex. We assessed multiple immunogen presentation formats, including monomeric, nanoparticulate, and transmembrane. We assessed antibody titers and their functionalities in traversal and standard membrane feeding assays, observing high titers and traversal inhibition from sera of vaccinated mice. We characterized cell-mediated immune responses by flow cytometry, investigating the populations of T_{FH}, T_{CM}, and T_{EM} cells induced. The biodistribution of circRNA-LNP vaccines was assessed to better understand the characteristics of effective circRNA-LNPs. Altogether, this work sets the stage for an effective multi-stage malaria vaccine and provides guidance for future work leveraging RNA vaccines against malarial pathogens.

6312

EFFECT OF MALARIA VACCINE CAMPAIGN IN REDUCING FEVER AMONG UNDER 24 MONTHS CHILDREN IN KEBBI STATE, NIGERIA

Michael Ekholuenetale[1], Oluwafunmilayo Ibikunle[1], Attahiru Muhammed Bello[1], Kolawole Maxwell[1], Nasiru Umar[2], Chuks Nnaji[3], Katherine Theiss-Nyland[4], Olusola Oresanya[1]

[1]Malaria Consortium, Abuja, Nigeria, [2]Malaria Programme, Kebbi State Ministry of Health, Nigeria, Kebbi, Nigeria, [3]Malaria Consortium, London, United Kingdom, [4]London School of Hygiene and Tropical Medicine, U. of London, London, United Kingdom

Kebbi State has one of the highest malaria prevalence rates in Nigeria. The Enhanced Seasonal Malaria Vaccination (ENHANCE) study was implemented in Kebbi State to improve malaria vaccine coverage among young children in two selected Local Government Areas (Birnin Kebbi and Koko/Besse). The study aimed to generate real-world evidence on strategies to optimise malaria vaccine coverage in highly seasonal transmission settings. The ENHANCE study assessed the effectiveness of a hybrid malaria vaccine delivery model using a three-arm nonrandomised controlled design with repeated cluster sampling at baseline and endline. Analytical methods included regression models estimating marginal effects on predicted fever probability. Fever probability was higher in areas where no malaria vaccine campaign occurred. In areas without a campaign, children who received the vaccine had a higher predicted probability of fever (~4-5%) compared with those who did not receive the vaccine (~3%), although confidence intervals were wide, indicating substantial uncertainty in this setting. In contrast, fever probability was lower in areas where a campaign occurred, with predicted fever probability at ≤1% in both groups. Children who received the vaccine had a slightly lower probability of fever than those who did not. These findings suggest that community-level vaccination campaigns may provide protective benefits beyond individual vaccination, potentially reflecting herd protection, improved awareness, or concurrent malaria control activities. Fever risk was highest in areas without a campaign, emphasising the importance of programmatic rollout and achieving high coverage alongside individual vaccination.

6313

PFRIPR5 BLOOD-STAGE MALARIA VACCINE CANDIDATE FORMULATED WITH TLR7 ADJUVANT SA-1 INDUCES POTENT FUNCTIONAL ANTIBODIES IN PRE-CLINICAL ANIMAL MODELS

Hikaru Nagaoka[1], Eizo Takashima[1], Ricardo Correia[2], Rute Castro[3], Tomomi Kakutani[4], Rieko Fujiwara[4], Masamitsu Aoki[4], António Roldão[2], Paula M. Alves[2], Akihisa Fukushima[4], Jorge Almeida[5], Candice Marion[5], Flavia Dalessio[5], Sophie Houard[5], Takafumi Tsuboi[6]

[1]Division of Malaria Research, Proteo-Science Center, Ehime University, Matsuyama, Japan, [2]iBET, Instituto de Biologia Experimental e Tecnológica / Instituto de Tecnologia Química e Biológica António Xavier, Universidade

Nova de Lisboa, Oeiras, Portugal, [3]iBET, Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal, [4]Vaccines Division, Sumitomo Pharma Co., Ltd, Osaka, Japan, [5]European Vaccine Initiative, Heidelberg, Germany, [6]Division of Cell-Free Sciences, Proteo-Science Center, Ehime University, Matsuyama, Japan

Two pre-erythrocytic malaria vaccines, RTS,S/AS01 and R21/Matrix-M, are currently being implemented in malaria-endemic countries in Africa. However, their efficacy is moderate, and durability needs improvement. Therefore, an additional asexual blood-stage vaccine that augments anti-disease immunity is required in addition to these first-generation pre-erythrocytic vaccines. The leading RH5-based blood-stage vaccine has demonstrated protective efficacy in malaria-endemic populations; however, its modest efficacy highlights RH5-interacting protein (PfRipr) as a promising additional blood-stage vaccine candidate. We previously reported that a functional fragment of PfRipr, PfRipr5, formulated with the CAF01 adjuvant, elicited rabbit antibodies with potent growth inhibition assay (GIA) activity. Here, to enhance the performance of the PfRipr5 vaccine, we formulated PfRipr5 with a novel TLR7 agonist adjuvant, SA-1, and evaluated its immunogenicity and GIA activity in rats. PfRipr5/SA-1 demonstrated higher immunogenicity and stronger functional activity than PfRipr5/CAF01. To prepare for a first-in-human trial, we also conducted a dose-selection study of both PfRipr5 and SA-1. Even low doses of SA-1 combined with PfRipr5 induced higher IgG levels than PfRipr5 alone. GIA activity in groups receiving a minimum effective dose of PfRipr5 with a low dose of SA-1 was significantly higher than in the PfRipr5-alone group, whereas antibody avidities were similar across groups receiving the same PfRipr5 dose irrespective of SA-1 dose. Notably, the PfRipr5/SA-1 formulation elicited a more pronounced Th1-type immune response than PfRipr5 alone. In summary, the high antibody titers, potent GIA activity, and Th1-biased responses induced by PfRipr5/SA-1 in rats, together with the establishment of GMP-compliant manufacturing processes for PfRipr5 and SA1, support the clinical development of PfRipr5/SA-1 as a next-generation asexual blood-stage *Plasmodium falciparum* malaria vaccine candidate.

6314

INTERIM RESULTS OF PHASE 2 STUDY OF PFS230D1-CRM197 WITH R21 IN MATRIX-M1, A MULTISTAGE *PLAMODIUM FALCIPARUM* VACCINE FOR MALARIA ELIMINATION, IN HEALTHY AFRICAN SCHOOL CHILDREN AND ADULTS

Julia Zemskova[1], Mahamadoun Hamady Assadou[2], Kourane Sissoko[2], Sadio D.K. Diarra[2], Seydou Sankare[2], Amagana Dolo[2], Aye Diallo[1], Alemush Imeru[1], Heather Ashley Goodman[1], Jen Hume[1], Daman Sylla[2], Justin Doritchamou[1], Amatigue Zeguime[2], Kelly M. Rausch[1], David L. Narum[1], Puthupparampil V. Scaria[1], Nicholas J. MacDonald[1], Daming Zhu[1], Agnes Guindo[2], Maria Conner[1], Rathy Mohan[1], Atul Karpe[3], Sandesh Madhukar Bharati[4], Dhananjay Kapse[3], Sara A. Healy[1], Umesh Shaligram[3], Prasad Kulkarni[5], Issaka Sagara[2], Patrick Duffy[1]

[1]National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States, [2]Malaria Research and Training Center, University of Sciences, Techniques and Technologies of Bamako, Bamako, Mali, [3]Serum Institute of India Pvt. Ltd., Pune, India, [4]Serum Institute of India Pvt Ltd, India, Pune, MH, India, [5]Serum Institute of India Pvt. Ltd., Bethesda, MD, United States

Progress in malaria control has stalled due to *Plasmodium falciparum* drug resistance, mosquito insecticide resistance, and socioeconomic barriers. The Laboratory of Malaria Immunology and Vaccinology and Serum Institute of India developed a multistage vaccine combining the transmission-blocking antigen Pfs230D1 with the licensed pre-erythrocytic vaccine R21 in Matrix-M1 to reduce both infections and transmission events. In a Phase 1 adult study, Pfs230D1-CRM197+R21 in Matrix-M1 was safe and immunogenic; the Phase 2 dose was chosen for highest antibody titers with minimal immune interference between vaccine components. This Phase 2 randomized, double-blind, controlled study evaluates safety, tolerability, immunogenicity, efficacy, and transmission-blocking activity of Pfs230D1-CRM197 with R21 in Matrix-M1. 1,200 participants (900 children 9-17 years; 300 adults 18-50 years) will be enrolled. IM vaccinations will be administered on days 0, 28, and 56 with either 6µg Pfs230D1-CRM197+10µg R21 in 50µg Matrix-M1 (co-formulated product), 10µg R21+50µg Matrix-M1, or rabies comparator. A subset of pediatric participants (n = 90) will receive a bedside formulation of the combination vaccine to compare antibody responses with the co-formulated product. Participants are followed for safety, clinical malaria (CM), and scheduled direct skin feeding assays (DSF) to measure transmission-blocking activity. Since September 2025, 219 adults have been enrolled; 198 have completed all three doses and remain on study. Thus far, we have observed 33 vaccine-related AEs and 10 DSF-related AEs, all mild-moderate (vaccine-related AEs: injection site pain as the most common local AE, while headache and fever being more common systemic AEs; DSF-related: local pruritus, pain, erythema); the study remains blinded as to treatment assignment. A futility analysis in adult participants enrolled in 2025 is being conducted to assess whether antibody responses to the co-formulated product are sufficiently high to justify proceeding with vaccination in remaining participants. Blinded safety data and overall study progress will be presented.

6315

A HISTORICAL PERSPECTIVE OF MILITARY MALARIA VACCINE DEVELOPMENT: WALTER REED ARMY INSTITUTE OF RESEARCH

Evelina Angov

Walter Reed Army Institute of Research, Silver Spring, MD, United States

For over four decades, Walter Reed Army Institute of Research (WRAIR) has been at the forefront of malaria vaccine development, driving critical scientific and clinical advancements. WRAIR's pioneering work established foundational tools, enabled the first licensed malaria vaccine, and continues to advance the development of globally effective, durable malaria vaccine strategies. A pivotal contribution from WRAIR was the development of the controlled human malaria infection (CHMI) model. This model is the global standard for the early evaluation of vaccine and drug efficacy. WRAIR's refinement of the CHMI model accelerated the clinical development of numerous antimalarial interventions. WRAIR played a vital role in the development of the RTS,S/AS01 malaria vaccine, the first to be licensed and recommended by the European Medicines Agency. Building on the experience with RTS,S, WRAIR engineered second-generation subunit vaccines. One example is the FMP013 malaria vaccine candidate, an optimized circumsporozoite protein (CSP) construct designed to expand epitope presentation and immunogenicity. This vaccine candidate has shown promising results, particularly when formulated with WRAIR's proprietary Army Liposome Formulation (ALFQ) adjuvants. ALFQ adjuvant has demonstrated potent immune-enhancing effects and is being evaluated in multiple vaccine trials. In parallel, WRAIR is developing structure-based nanoparticle vaccines. These innovative platforms display malaria antigens in highly ordered, multivalent arrays to boost B cell activation and elicit potent antibody responses. This approach overcomes the limitations of subunit vaccines by improving the magnitude and quality of immune responses. More recently, WRAIR expanded its research to next-generation platforms, including mRNA. Preclinical studies of mRNA-based malaria vaccines have shown promising results, demonstrating the potential for rapid development and induction of robust immune responses. These efforts are part of a broader strategy to leverage cutting-edge technologies to accelerate the development of globally effective malaria vaccines.

6316

PROTECTIVE CELLULAR IMMUNE RESPONSES FOLLOWING IMMUNIZATION WITH NON-REPLICATING AND REPLICATING PFSPZ VACCINES AND CONTROLLED HUMAN MALARIA INFECTION

Lauren P. Jatt[1], Sean C. Murphy[2], Stephen L. Hoffman[3], Stephen C. De Rosa[1], James Kublin[1]

[1]Fred Hutch, Seattle, WA, United States, [2]Univ of Washington, Seattle, WA, United States, [3]Sanaria Inc, Rockville, MD, United States

Malaria remains a major global health threat, yet there is still no highly efficacious and durable vaccine. A major barrier to malaria vaccine development is the absence of an established immunologic correlate of protection for sterilizing immunity. Controlled human malaria infection (CHMI) trials provide a rare opportunity to directly link immune responses with a clinically meaningful efficacy endpoint, because vaccinated participants subsequently undergo CHMI and are then monitored for parasitemia. To date, no robust and reproducible correlates of protection have been identified for malaria vaccination. Evidence from animal models and human studies suggests that cellular, rather than humoral, immune responses are central to sterilizing immunity, but the relevant protective populations remain undefined. Prior attempts to confirm cellular correlates of protection have failed in part due to small sample sizes in individual trials and the lack of standardization of cellular assays across trials. To address the lack of standardization of cellular assays, the Fred Hutch cellular laboratory of the HIV Vaccine Clinical Trials Network Laboratory Center developed validated high-dimensional flow cytometry assays with rigorous quality assurance and quality control measures that ensure reproducible and consistent results across trials. Using this validated high-dimensional flow cytometry assay, we characterized phenotypic and functional cellular immune responses from 29 protected and 28 infected participants from two prior CHMI trials of radiation-attenuated *Plamodium falciparum* sporozoite (PfSPZ) vaccine and chemo-attenuated PfSPZ-CVac (chloroquine). Protected vaccinees had significantly higher levels of polyfunctional malaria specific CD4 T cells expressing IFN**γ**;-IL2-TNFα. Full results from these assays, including additional potential cellular correlates of protection, will be presented.

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HOLLOW MICRONEEDLE-BASED INTRADERMAL DELIVERY ENABLES HIGHLY PROTECTIVE HETEROLOGOUS VIRAL VECTOR MALARIA VACCINATION

Satoshi Benoki[1], Yuta Suzuki[1], Yui Miura[2], Masaki Yamamoto[1], Yuna Sato[2], Yutaro Yamamoto[2], Ryota Watano[3], Hisatoshi Shida[4], Hiroaki Mizukami[3], Shigeto Yoshida[2], Takuya Mori[1]

[1]Biological and Material Science Research, Kao Corporation, Haga-Gun Ichikai-Machi, Japan, [2]Laboratory of Vaccinology and Applied Immunology, Kanazawa University School of Pharmacy, Kanazawa, Japan, [3]Division of Genetic Therapeutics, Center for Molecular Medicine, Jichi Medical University, Shimotsuke, Japan, [4]Laboratory of Primate Model, Research Center for Infectious Diseases, Institute for Frontier Life and Medical Science, Kyoto University, Kyoto, Japan

Malaria remains a major global health burden, and vaccines with improved efficacy and durability are needed. We previously developed a heterologous prime-boost malaria vaccine platform using the attenuated vaccinia virus strain LC16m8Δ (m8Δ) for priming and adeno-associated virus serotype 8 (AAV8) for boosting, which achieved high protection in mice when administered via tail scarification (t.s.) followed by intravenous (IV) injection. However, IV administration is invasive and impractical for large-scale vaccination programs. To address this limitation, we evaluated intradermal (ID) delivery using a hollow microneedle device developed by Kao Corporation as a minimally invasive alternative route. Mice were immunized with an m8Δ/AAV8 vaccine encoding pre-erythrocytic (PE) and liver-stage (LS) antigens using three regimens: the conventional t.s./IV regimen, ID/IV and t.s./ID. Protective efficacy was evaluated by IV challenge with Pb (PE^-/LS^+) sporozoites. At the first challenge, protection rates were 93% in the conventional t.s./IV group, 80% in the ID/IV group, and 70% in the t.s./ID group, while naïve controls showed no protection. All vaccinated groups demonstrated significantly higher protection than naïve mice, and no statistically significant differences were observed among the vaccinated regimens. Protective efficacy was maintained at the second challenge performed 11 weeks later. Although PE-specific antibody titers were lower in ID-containing regimens than in the conventional group, substantial protection was maintained, suggesting an important contribution of LS-specific cellular immunity. We hypothesize that the superior efficacy observed with IV boosting is mediated by efficient hepatic delivery of AAV8-LS, leading to the induction of LS-specific liver-resident memory $CD8^+$ T cells that mediate sterile protection against sporozoite infection. These findings indicate that microneedle-based intradermal delivery represents a minimally invasive and clinically feasible alternative administration route for the m8Δ/AAV8 malaria vaccine platform while maintaining high protective efficacy.

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SAFETY OF THE RTS,S/AS01E MALARIA VACCINE UP TO ONE YEAR AFTER THE FULL-SCHEDULE COMPLETION IN REAL-LIFE SETTINGS IN THREE SUB-SAHARAN AFRICAN COUNTRIES: FINAL ANALYSIS

Bernhards Ogutu Ragama[1], and the RTS,S Epidemiology EPI-MAL-003 study group[2]

[1]Kenya Medical Research Institute, Centre for Clinical Research, Kisumu, Kenya, [2]RTS,S Epidemiology EPI-MAL-003 study group

In 2021, WHO recommended widespread use of RTS,S/$AS01_E$ for children from 5 months of age living in areas with moderate to high transmission of *Plasmodium falciparum*. The EPI-MAL-003 (NCT03855995) surveillance study with prospective cohort event monitoring, involving home visits, outpatient visits and hospitalizations, was conducted within the framework of the WHO-coordinated Malaria Vaccine Implementation Programme in Ghana, Kenya and Malawi. The study aimed to evaluate the safety signals identified in the RTS,S/$AS01_E$ phase 3 trial (NCT00866619). Safety outcomes were compared between RTS,S/$AS01_E$-vaccinated children under 5 years of age from exposed clusters (where RTS,S/$AS01_E$ had been introduced) and unvaccinated children from unexposed clusters (where the vaccine had not been introduced). We report here incidence rates (IRs) per 100,000 person-years of meningitis, malaria and mortality, up to one year after the full vaccination schedule (3+1 doses). This final analysis included 22,448 children in exposed and 22,464 in unexposed clusters. The fourth RTS,S/$AS01_E$ dose coverage was 64.3% in the exposed clusters. Etiology confirmed meningitis was reported in 1 vaccinated (IR: 2.6, 95% confidence interval [CI]: 0.1-14.2) and 1 unvaccinated (IR: 2.4, 95% CI: 0.1-13.4) child. Cerebral malaria cases were reported for 5 vaccinated (IR: 12.8, 95% CI: 4.1-29.8) and 7 unvaccinated (IR: 16.8, 95% CI: 6.8-34.6) children. All-cause mortality was reported for 197 vaccinated (IR: 502.5, 95% CI: 434.8-577.8) and 226 unvaccinated (IR: 542.9, 95% CI: 474.4-618.5) children, with similar IRs observed in vaccinated girls (IR: 497.7, 95% CI: 403.2-607.8) and boys (IR: 507.1, 95% CI: 413.0-616.2). Meningitis and cerebral malaria were rare with similar distribution among vaccinated and unvaccinated children. Mortality rates were comparable between boys and girls. Up to 1 year after the full-schedule vaccination, RTS,S/$AS01_E$ was not associated with any of the safety signals previously observed in the phase 3 trial. Overall, the benefit-risk profile of RTS,S/$AS01_E$ remained favorable.

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DEVELOPMENT OF A MULTIVALENT MRNA-BASED VACCINE FOR *PLAMODIUM FALCIPARUM* MALARIA THAT TARGETS MULTIPLE STAGES TO ACHIEVE HIGH EFFICACY

Chetan E. Chitnis[1], Alberto Ayllon-Hermida[1], Eric Legrand[1], Gracie H. Amourda[1], Christele Huon[1], Rebecca Lampley[2], Hamilton Bennett[2], Jennifer Pham[1], Anthony Bouillon[1], Sabine Thiberge[1]

[1]Institut Pasteur, Paris, France, [2]Moderna Tx, Cambridge, MA, United States

Plasmodium falciparum malaria remains a major public health problem in many parts of the tropical world. Despite continued implementation

of malaria control efforts, malaria incidence has remained unchanged over the past 10 years. The availability of high efficacy vaccines for *P. falciparum* malaria could greatly augment our efforts to eliminate malaria. We propose to develop mRNA-based vaccines that target *P. falciparum* pre-erythrocytic (PE) stage sporozoites and blood stage merozoites. We have developed mRNA vaccine constructs based on the validated PE vaccine antigen, PfCSP. mRNA constructs for PfCSP fused to hepatitis B surface antigen (HBsAg) (HBsAg-PfCSP) formulated in lipid nano-particles (LNPs) elicit potent antibody responses in C57BL/6 mice that provide high levels of sterile protection against challenge with transgenic *P. berghei* sporozoites expressing PfCSP (Pb-PfCSP spzs). In addition, we have developed a mRNA vaccine construct, PfRH5-TM, that expresses PfRH5 on the mammalian cell surface. Immunization with PfRH5 elicits potent growth inhibitory antibodies that target PfRH5 to block adhesion to its receptor basigin on RBCs. Immunization with the combination of HBsAg-PfCSP and PfRH5-TM elicits balanced antibodies against both liver and blood stage parasites that protect against sporozoite challenge and block blood stage growth. These studies establish the feasibility of developing multicomponent, mRNA-based vaccines that target both pre-erythrocytic and blood stage parasites with the goal of achieving high efficacy.

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HIGH ESBL AND CARBAPENEM RESISTANCE IN NORTHERN GHANA: IMPLICATIONS FOR STEWARDSHIP AND ACCESS TO EFFECTIVE ANTIBIOTICS

Amos A. Bugri[1], Jack G. Schneider[2], Laud Boateng[3], Samuel Alomatu[3], David E. Zeyeh[3], Clement Amedor[4], Thomas Duszynski[1], Debra Litzelman[5], Rajesh Vedanthan[6], Akosua Bonsu[4], Adriana Gardner[7], Angelica Kottkamp[6]

[1]Indiana University, Richard M. Fairbanks School of Public Health, Indianapolis, IN, United States, [2]Indiana University School of Medicine, Indianapolis, IN, United States, [3]Tamale Teaching Hospital, Tamale, Ghana, [4]University for Development Studies, UDS, Tamale, Ghana, [5]Indiana University Center for Global Health, Indianapolis, IN, United States, [6]NYU Grossman School of Medicine, New York, NY, United States, [7]Purdue University College of Pharmacy, West Lafayette, IN, United States

Antimicrobial resistance (AMR) is a major global health threat that disproportionately affects sub-Saharan Africa. Although studies from Ghana report high resistance rates, laboratory capacity and surveillance remain limited, hindering evidence-based antimicrobial prescribing. Tamale Teaching Hospital (TTH), the largest tertiary referral center in northern Ghana, recently expanded its clinical microbiology laboratory, enabling routine antimicrobial susceptibility testing. We conducted a retrospective analysis of microbiologic culture results from 2019-2024, excluding wound cultures. Minimum inhibitory concentrations were interpreted using CLSI guidelines, and extended-spectrum β-lactamase (ESBL) production was defined as *Enterobacterales* resistant to ceftriaxone. Pathogen identification, specimen source, and susceptibility data were captured using WHONET and REDCap. Descriptive statistics summarized pathogen distribution and resistance patterns. Among 1,300 isolates, 961 (73.9%) were Gram-negative bacteria, 205 (15.8%) Gram-positive bacteria, and 134 (10.3%) yeast. The most common pathogens were *Citrobacter* spp. (22.0%), *Klebsiella* spp. (20.8%), *Escherichia coli* (12.0%), and *Candida* spp. (10.3%). Most specimens were from urine (n=734, 56.5%) and blood (n=156, 12.0%). Among *Enterobacterales* tested, 83.8% (337/402) produced an ESBL and 44.7% (71/159) showed carbapenem resistance. Trimethoprim-sulfamethoxazole resistance among *Staphylococcus aureus* isolates (n=128) was 70%. Overall, high rates of ESBL-producing and carbapenem-resistant organisms were identified, informing empiric therapy and antimicrobial stewardship efforts and highlighting the need for access to agents active against these pathogens at TTH. Notably, *Citrobacter* spp. was the most common pathogen, unlike most AMR datasets where *E. coli* and *Klebsiella* spp predominate. Continued surveillance will be important for monitoring resistance trends and guiding antimicrobial policy in northern Ghana.

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ANTIBIOTICS AND PRIORITY EFFECTS IN COLONIZING RESISTANT *ESCHERICHIA COLI* STRAIN ASSEMBLAGES IN COMMUNITY SETTINGS

Eric Ng'eno

University of Kansas, Lawrence, KS, United States

Colonizing resistant bacteria often exist within functionally structured microbial communities, where members' occurrences are influenced by their interactions. Considering these interactions could improve understanding of factors influencing the diversity of colonizing antibiotic resistant bacteria within human community settings. Using a framework that implicitly considered biotic interactions, we investigated factors associated with assemblage of colonizing antibiotic-resistant *Escherichia coli* strains in a densely populated urban informal settlement. We identified antibiotic selection, colonization legacy, positive feedbacks and dispersal as key assembly processes. Antibiotics and colonization legacy exerted strain-specific effects while dispersal factors influenced all strains rather uniformly. Specifically, areas frequently exposed to bactericidal antibiotics were dominated by locally established resistant strains, whereas areas frequently exposed to bacteriostatic antibiotics had increased presence of less-established strains. Assemblage configuration was determined by identity and relative abundance of strains initially present at each locality. Hygiene practices reinforced local strains consistently while weather variables had variable effects: high monthly average minimum temperatures increased abundance of nearly all strains, whereas increases in monthly average maximum temperature, humidity, and rainfall uniformly decreased strain abundance. Overall, we show that survival selection, driven by selective forces, and dispersal selection, shaped by colonization potential, jointly influenced recolonization and reconfiguration of resistant bacterial communities, suggesting antibiotics and priority effects as key determinants of resistant *E. coli* assemblages in this setting. Together these findings underscore need for context-specific antibiotic stewardship and hygiene interventions.

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ECONOMIC BURDEN OF CARBAPENEM-RESISTANT *KLEBSIELLA PNEUMONIAE* INFECTIONS IN NEPAL AND BHUTAN

Yunjin Yum[1], Andreia Santos[1], Wongyeong Choi[1], Sonam Wangda[1], Geun Hyeog Jang[1], Saehyeun Song[1], Pradip Mishra[2], Sonam Zangmo[3], Dilip Sharma[4], Nimesh Poudyal[1], Florian Marks[1], Jung-Seok Lee[1]

[1]International Vaccine Institute, Seoul, Republic of Korea, [2]Seti Provincial Hospital, Dhangadhi, Nepal, [3]Jigme Dorji Wangchuck National Referral Hospital, Thimphu, Bhutan, [4]Bir Hospital, Kathmandu, Nepal

Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) is an emerging public health threat associated with poor clinical outcomes. South Asia experiences one of the highest mortality burdens related to *Klebsiella pneumoniae* infections, yet evidence on the economic impact of CRKP remains limited in low- and middle-income countries (LMICs). This study aimed to estimate the economic burden of CRKP infections using patient-level cost-of-illness data from Nepal and Bhutan and to compare costs across health systems with different financing structures. A multicenter cohort study was conducted between May and December 2025 in three hospitals across the two countries. Patients with laboratory-confirmed infections and antimicrobial susceptibility results were categorized into three cohorts: CRKP (Cohort 1), carbapenem-susceptible *K. pneumoniae* (CSKP; Cohort 2), and non-*K. pneumoniae* infections (Cohort 3). Patient-level costs—including direct medical, direct non-medical, and indirect costs—were collected through structured interviews, while public healthcare expenditures and clinical information were obtained from hospital records. To address potential confounding, inverse probability weighting, instrumental variable approaches, and generalized linear models were used. In both Nepal and Bhutan, the mean total cost per episode was higher for CRKP infections than for CSKP infections and non-*K.*

pneumoniae infections. Public healthcare expenditures accounted for the majority of costs in Bhutan due to the publicly financed health system. After adjustment, CRKP infections were consistently associated with higher costs than both CSKP and non-*K. pneumoniae* infections. Final analyses will be completed prior to the ASTMH meeting, and the complete results will be presented at the conference. These findings provide robust evidence on the economic impact of CRKP infections in LMICs, supporting standardized comparisons across health priorities and informing decision-making while highlighting the need to strengthen antimicrobial resistance control.

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ECONOMIC BURDEN OF INFECTIONS WITH ANTIMICROBIAL RESISTANCE IN LAOS

Wongyeong Choi[1], Phonevilai Santisouk[1], Yunjin Yum[1], Jaewoong Lee[1], Saehyeun Song[1], Palada Souvanhnavong[1], Khaennakhone Salodchanar[1], Naphaphone Khaththiyavong[2], Soulivanh Phanthavong[2], Loungnilanh Manivanh[2], Vangnakhone Dittaphong[2], Jung-Seok Lee[1]

[1]International Vaccine Institute, Seoul, Republic of Korea, [2]Setthathirath Hospital, Vientiane, Lao People's Democratic Republic

Antimicrobial resistance poses a major global health threat, yet its economic impact on low- and middle-income countries is poorly understood. This study estimated the economic burden of infections caused by extended-spectrum beta-lactamase producing (ESBL-producing) *Escherichia coli* (*E. coli*), an important resistant pathogen, in Laos. A prospective cost-of-illness study was conducted among patients with laboratory-confirmed infections in Setthathirath Hospital in Vientiane, Laos. Clinical and cost data were collected through repeated patient interviews and medical record review**.** Econometric modeling approaches, including inverse probability weighting (IPW) and instrumental variable (IV) analyses, were applied to estimate out-of-pocket expenditures, public expenditures, total cost of illness, and length of stay, while accounting for potential selection bias. Direct medical costs accounted for the largest share across cohorts, while direct non-medical and indirect costs also contributed a substantial proportion. Findings were consistent across multiple analytic approaches, including unadjusted, IPW-adjusted, and IV analyses. Overall, ESBL-producing *E. coli* infections were associated with substantially higher economic burden compared with other groups. These results indicate that ESBL-producing *E. coli* infections impose a considerable economic burden in Laos and underscore the importance of strengthened antimicrobial stewardship, infection prevention, and improved diagnostic capacity.

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BLACK SEED OIL INHIBITS GROWTH OF SEVERAL BACTERIA SPECIES

James N. Mubiru[1], Hanh Nguyen[2]

[1]College of the Mainland, Texas City, TX, United States

The development of microbial resistance has become a global challenge because it has rendered standard treatments ineffective, threatening to return medicine to a pre-antibiotic era. Black seed (*Nigella sativa*) oil has traditionally been used as herbal remedy against several conditions in the Middle East and many Asian countries. Recent studies have shown its effectiveness against several bacteria species. We set out to investigate the inhibitory effects of store-bought black seed oil with a 1.2% thymoquinone concentration on several bacteria species. We carried out the Kirby-Bauer test and the minimum inhibitory test on several bacteria species. Our results show that black seed oil inhibits the growth of *Klebsiella pneumoniae*, *Micrococcus luteus, Bacillus cereus, and Staphylococcus aureus*. On the Kirby-Bauer test black seed oil showed very high inhibition zones against *Bacillus cereus*. The minimum inhibitory concentration of black seed oil was 0.2% The bacteria which were inhibited by black seed oil are all known to cause infections in humans, particularly when host defenses are compromised. These bacteria are known for their ability to acquire resistance to various antibiotics, making treatment challenging**.** They are also capable of forming biofilms on surfaces, including medical devices which contribute to their persistence and resistance to treatment. Black seed oil has a long history of use in traditional medicine and has few side effects. Further studies are needed to explore the possibility of clinical usage of black seed oil on antibiotic resistant bacteria like MRSA.

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EPIDEMIOLOGY AND TRANSMISSION OF HEALTHCARE-ASSOCIATED INFECTIONS AND ANTIMICROBIAL RESISTANCE IN A TERTIARY HOSPITAL, YAOUNDE, CAMEROON, 2025

Bayong Kyeng Mirabelle[1], Kamga H. Hortense[2], Jane F. Akoachere[3], Darlingtine E. Ngale[4], Dickson S. Nsagha[5]

[1]Department of Public Health & Hygiene, Faculty of Health Sciences, University of Buea, Cameroon, [2]Faculty of Medicine and Biomedical Sciences, University of Yaounde 1, Yaounde, Cameroon, [3]Faculty of Sciences, University of Buea, Cameroon, [4]Catholic University of Central Africa, School of Health Sciences, Yaoun, Cameroon, [5]Department of Public Health & Hygiene, Faculty of Health Sciences, University of Buea, Cameroon

The risk of healthcare-associated infections (HAIs) and antimicrobial resistance (AMR) is often exacerbated by prolonged hospitalization. HAIs and AMR remain leading causes of morbidity and mortality worldwide. This study aimed to describe the epidemiology and transmission dynamics of HAIs/AMR pathogens within the hospital environment. We conducted two Point Prevalence Surveys (PPS) during the last weeks of July and October 2025 across 05 units of the Yaoundé University Teaching Hospital (YUTH). We used a contextualized version of the European CDC PPS protocol and the Cameroon HAI surveillance guidelines. We collected biological samples from patients suspected of having HAI and from their environments (bed, bedside cupboard, water source, air). Standard microbiological methods for pathogen identification and antimicrobial susceptibility testing employed. Data analyzed using EPI Info 7.2.5.0 and Microsoft Excel 2016. Descriptive and comparative analysis done with statistical significance set at $p<0.05$ and 95% C.I. Fourteen out of the sixty-nine hospitalized patients had HAI, a prevalence of 20%. 57% (8/14) had respiratory associated infections, 43% (6/14) had catheter associated urinary tract infection. Hospitalization units implicated were surgical reanimation and medical. Male-female sex ratio 1:1, median age 53 (03 to 75 years), average hospital stay:19 days. Broad-spectrum antibiotics (carbapenems and glycopeptides) were frequently prescribed. Predominant pathogen identified *was Klebsiella pneumoniae* (50%). Multidrug resistance observed in *Klebsiella. pneumoniae, Pseudomonas aeruginosa and Acinetobacter baumannii*. A significant association between *Klebsiella pneumoniae* isolates from patients and the environment ($p = 0,019$). Strong correlations in antimicrobial resistance profiles between patient and environmental isolates also identified ($r = 0,791$; $p = 0,011$). HAI/AMR pathogens in patients and immediate environment suggest environmental role in transmission. The need to reinforce infection prevention and antimicrobial stewardship in hospitals to limit the occurrence of HAIs and AMR.

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COMPARATIVE PERFORMANCE OF PORTABLE DNA EXTRACTION PROTOCOLS AND BIOINFORMATICS WORKFLOWS FOR RAPID DETECTION OF PATHOGENS AND ANTIMICROBIAL RESISTANCE USING OXFORD NANOPORE SEQUENCING

Louis Kyei-Tuffuor[1], Samuel K. Agordzo[2], Angela K. Asante[2], Davina S. Boateng[2], Winifred N. S. Adjei[2], Venus N. B. Frimpong[2], Rita Nartey[2], Francis A. Yeboah[1], Rea M. Kobialka[3], Ahmed Abd El Wahed[3], Uwe Truyen[3], Yaw A. Amoako[2], Richard O. Phillips[2], Michael Frimpong[2]

[1]Department of Molecular Medicine, School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, [2]Kumasi

Centre for Collaborative Research in Tropical Medicine, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, [3]Institute of Animal Hygiene and Veterinary Public Health, Leipzig University, Leipzig, Germany

Oxford Nanopore sequencing (ONS) holds immense potential for decentralized antimicrobial resistance (AMR) surveillance, but the quality of DNA extraction heavily influences the reliability of downstream genomic analyses. This study evaluated four portable bacterial DNA extraction protocols to determine their impact on sequencing performance, bioinformatics completion, and field-deployability in low-resource settings. Six Gram-negative isolates (*E. coli*, n=4; *Pseudomonas* sp., n=1; *Salmonella* sp., n=1) were processed using SwiftX DNA, SwiftX DNA with Proteinase K, SwiftX ParaBact, and NucleoSpin Microbial extraction protocols. Twenty-four DNA extracts were multiplexed on a single MinION R10.4.1 flow cell. Data were analyzed using Galaxy-based bioinformatics pipelines, with workflow completion defined as successful progression through quality control, assembly, virulence profiling, plasmid, and AMR gene detection modules. DNA purity varied substantially across protocols and was strongly associated with workflow success (Spearman's ρ=0.767, p<0.0001). NucleoSpin Microbial achieved 100% workflow completion, and SwiftX ParaBact achieved 83%, whereas both SwiftX DNA protocols failed to complete full workflows. Importantly, key AMR genes required to classify isolates as multidrug-resistant were consistently detected with both NucleoSpin Microbial and SwiftX ParaBact protocols. However, NucleoSpin Microbial-derived assemblies showed significantly higher contiguity and enabled broader detection of virulence factors, pathogenicity islands, plasmid replicons, and accessory AMR genes. In conclusion, the choice of DNA extraction method fundamentally dictates ONS success. While silica-column extraction maximized genomic completeness, paramagnetic bead-based reverse purification (SwiftX ParaBact) offered superior portability with sufficient resolution for frontline AMR surveillance. These findings highlight a practical trade-off between field deployability and high-resolution genomic characterization in low-resource settings.

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ANTIMICROBIAL RESISTANCE IN *SHIGELLA* ISOLATES IN LATIN AMERICA: A SYSTEMATIC REVIEW AND META-ANALYSIS

Javier Cabrera-Sanchez[1], **Carolina Ruiz-Mercado**[1], Jorge Alave[2]
[1]Facultad de Medicina, Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Escuela de Medicina, Universidad Peruana Union, Lima, Peru

Shigella is a leading cause of diarrheal disease globally, particularly in low-income countries. However, antimicrobial resistance patterns across Latin America have not been comprehensively synthesized. We conducted a systematic review and meta-analysis to evaluate antimicrobial resistance to first-line antibiotics in the region. We searched PubMed, Scopus, LILACS, and SciELO for studies published between January 1995 and June 2025, with no language restrictions, reporting the prevalence of antimicrobial resistance in *Shigella* isolates from patients with acute diarrhea in Latin American countries. We conducted random-effects meta-analyses to estimate the pooled country-specific prevalence of resistance for each first-line antibiotic. Heterogeneity was quantified using the I^2 statistic (PROSPERO CRD420261336385). We screened 406 abstracts, retrieved 150 full-text articles for eligibility, and included 56 studies involving 15,446 *Shigella* isolates. Twelve countries contributed at least 2 studies to the meta-analysis (Argentina, Brazil, Chile, Costa Rica, Cuba, Ecuador, Guatemala, Mexico, Peru, Trinidad, Uruguay, Venezuela). Comparisons of pooled antibiotic resistance showed that ampicillin resistance was high across most countries, exceeding 60% in several settings, and ranged from 15.3% in Trinidad (95% CI 11.2-20.6; 2 studies, n=425) to 80.2% in Peru (95% CI 69.3-87.9; 5 studies, n=2985). Similarly, high resistance to trimethoprim-sulfamethoxazole was seen in most countries (12.3% to 82.2%). Pooled resistance to ceftriaxone and ciprofloxacin was consistently low across countries, not exceeding 2.1% and 1.7%, respectively. Between-study heterogeneity was high. Azithromycin resistance data were scarce. Our analysis shows that antimicrobial resistance to ampicillin and trimethoprim-sulfamethoxazole among *Shigella* isolates in Latin America is high and varies by country. Resistance to ceftriaxone and ciprofloxacin remains low. These results underscore the need for ongoing surveillance of first-line antibiotic resistance and support further studies in countries with limited data.

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COMPARATIVE ANALYSIS OF CONVENTIONAL BACTERIOLOGY AND NEXT-GENERATION SEQUENCING FOR PATHOGEN IDENTIFICATION AND ANTIMICROBIAL RESISTANCE DETECTION IN HEALTHCARE-ASSOCIATED INFECTIONS IN INTENSIVE CARE UNITS IN SENEGAL

Fall Awa
Cheikh Anta Diop University (UCAD), Dakar, Senegal, Dakar, Senegal

Healthcare-associated infections (HAIs) represent a major public health challenge, particularly in intensive care units (ICUs), where antimicrobial resistance (AMR) is highly prevalent. This study aimed to evaluate the contribution of next-generation sequencing (NGS) compared with conventional bacteriology for bacterial identification and characterization of antimicrobial resistance mechanisms. A prospective study was conducted between March 2023 and April 2024 at the bacteriology laboratory of the National Hospital Center Dalal Jamm (CHNDJ), Dakar, Senegal. Bacterial isolates obtained from ICU patients with HAIs were analyzed using conventional bacteriology and NGS. Bioinformatic analyses were performed using the CZ ID platform**,** and resistance gene heatmaps were generated with GuideTAPE. Among the 18 isolates analyzed, 61% showed concordance between conventional bacteriology and NGS, while 39% exhibited partial or complete discordance. NGS provided higher taxonomic resolution, enabled the detection of co-infections, and identified a wide range of antimicrobial resistance genes, including rare or emerging determinants. In conclusion, NGS represents a powerful complementary tool to conventional microbiology, enhancing bacterial identification and strengthening antimicrobial resistance surveillance in intensive care settings.

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LABORATORY VALIDATION OF THE MA120 COLORIMETRIC IDENTIFICATION AND BROTH MICRODILUTION AST PLATFORM IN URBAN MALAWI

Casey Vieni[1], Alick Misanjo[2], Tadala Rambiki[2], Tiffany Priester[3], **Matthew Cappiello**[4]
[1]Mayo Clinic, Rochester, MN, United States, [2]Blantyre Adventist Hospital, Blantyre, Malawi, [3]Jerry L Pettis Memorial Veterans Hospital, Loma Linda, CA, United States, [4]Loma Linda University Medical Center, Loma Linda, CA, United States

Accurate lab testing guides treatment and prevents antimicrobial resistance. Current AST workflows at Blantyre Adventist Hospital in Malawi use Kirby-Bauer discs, which require technologist input with extraneous AST testing in 42% of cases. For semi-automated organism ID and antibiotic susceptibility testing (AST), BAH acquired a Chinese-manufactured MA120 (Render Biotech Co). Data are limited regarding performance characteristics. Validation of Enterobacteriaceae, Nonfermenter, Staphylococcus, and Strep/Enterococcus kits for the MA120 were run according to manufacturer instructions. Protocols were undertaken according to provided SOP. MA120 output was compared to manual interpretation of microtiter plates per Clinical and Laboratory Standards Institute (CLSI) M07. MA120 software correctly reported positive growth control in 8/14 (57%) samples. Automatic reads identified the correct organism 66.6% of the time. 41.6% of isolates were correctly identified with software-assisted interpretation, and 34.2% of isolates were correctly identified if there was a deviation from manufacturer SOP. The MA120 reported organism identification 100% of the time, even if automatic read of the positive growth control was negative, post-incubation reagents were not added, incorrect kit was used, or plates were not physically loaded in the instrument. The precision of the MA120 was 100% for the same plate/same isolate. Essential agreement (EA) between the same isolates varied 22.2-96.0% depending on kit and method, with >90% categorical agreement (CA) and EA for the Staph kit. EA and CA were 25-30% lower when using the incorrect kit. Fungal kit could not be validated

due to absence of reference strains and alternative testing methods, and unavailability of manufacturer SOPs. This work highlights the challenges of performing instrument validation in low-resource settings, as well as the need for careful validation of foreign-manufactured equipment before clinical utilization. The MA120 in this setting could not reliably identify or perform AST for common organisms. Use of the device was found to be fraught with potential for error.

6329A

MOLECULAR DETECTION OF AMBLER CLASSES A AND C β-LACTAMASES RESISTANCE GENES IN VIBRIO CHOLERAE AND VIBRIO PARAHAEMOLYTICUS FROM AQUACULTURE CLUSTERS IN TWO SOUTHERN STATES IN NIGERIA

S. A. Alarape, O. A. Odeniyi, O. K. Adeyemo
University of Ibadan, Ibadan, Nigeria

The global rise in antibiotic-resistant bacteria poses a serious threat to public health, especially in aquatic environments where *Vibrio* species are widespread and linked to foodborne diseases. *Vibrio cholerae* and *Vibrio parahaemolyticus* are common fish pathogens, and their ability to harbour β-lactamase resistance genes raises concerns about the potential spread of resistance to other bacteria, including those infecting humans. Liver, kidney and spleen were collected from adult fish (n=285). Sampled tissues were enriched in buffered peptone water and incubated at 30℃ for 24 hours, followed by culture on Thiosulfate Citrate-Bile salts Sucrose (TCBS) Agar. Colonies were identified based on morphology and biochemical tests. Real-time PCR (NZYtech) and whole-genome sequencing were used to confirm species identity and detect β-lactamase resistance genes. *V. cholerae* appeared as yellow, round colonies with smooth edges, while *V. parahaemolyticus* formed green colonies with similar morphology. *Vibrio parahaemolyticus* showed a typical sigmoid curve in RT-PCR, while *V. cholerae* did not. Genomic analysis revealed that *V. cholerae* carried the *HugA* gene, an Ambler class A β-lactamase. *V. parahaemolyticus* harboured multiple resistance genes, including Ambler class A β-lactamases (*blaTEM-1, blaLAP-2, HugA*) and class C β-lactamases (*blaACT-41, blaMIR-14*). Other resistance genes detected included those conferring resistance to aminoglycosides [*aac(6')-Iid, aadA1, aadA2, aph(3")-Ib, aph(6)-Id*], chloramphenicol (*catA4*), trimethoprim (*dfrA1, dfrA14*), florfenicol (*floR*), fosfomycin (*fosA*), quinolones (*oqxA, oqxB9, qnrD1, qnrS1*), streptothricin (*sat2_gen*), sulfonamides (*sul2*), and tetracyclines [*tet(57), tet(59), tet(H), tet(J)*]. The presence of multidrug resistance genes, particularly β-lactamases, in *Vibrio* species from aquaculture environments, underscores the risk of resistance gene transfer and potential public health implications. Continuous monitoring of antibiotic use and resistance in aquaculture is critical for safeguarding animal, human, and environmental health within a One Health framework.

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TRANSIENT SMALL BOWEL INTUSSUSCEPTION AS AN UNUSUAL MANIFESTATION OF PROBABLE ENTERIC FEVER IN A CRUISE SHIP WORKER

Kana Satake, Sanah Siddiqui, Genevieve Hughes, Natalie Cedeno, David C. Perlman
Mount Sinai West, Icahn School of Medicine at Mount Sinai, New York, NY, United States

A 26-year-old man from Indonesia presented with four days of high fever, watery non-bloody diarrhea, nausea, and intermittent vomiting. He had worked aboard a commercial cruise ship for three weeks without disembarking and had received intravenous ceftriaxone onboard without improvement. On admission, he was febrile to 40.2°C with mild right upper quadrant tenderness and scleral icterus. Laboratory testing showed a normal leukocyte count (6.9 × 10^9/L) and marked transaminitis (AST 498 U/L, ALT 365 U/L, alkaline phosphatase 291 U/L). Abdominal and pelvic computed tomography revealed a small bowel-to-small bowel intussusception in the left upper abdomen without obstruction or ischemia, as well as a small splenic infarct. The intussusception appeared benign on imaging and was managed conservatively. He was treated with ceftriaxone, but fevers persisted for five days after admission. Stool multiplex PCR was positive for *Salmonella* species and negative for other enteric pathogens; blood cultures and testing for malaria, viral hepatitis, EBV, and HIV were negative. Persistent high fevers despite antimicrobial therapy, marked transaminitis, splenic infarction, and epidemiologic risk raised concern for enteric fever, although species-level confirmation was limited by negative cultures. Repeat CT two days later demonstrated complete resolution of the intussusception without intervention. Enteric fever is endemic in South and Southeast Asia, and gastrointestinal complications more commonly include hemorrhage or perforation, whereas intussusception is rarely described. This case highlights transient adult small bowel intussusception as an unusual clinical and radiologic manifestation of suspected invasive *Salmonella* infection and underscores the importance of considering enteric fever in febrile travelers or globally mobile workers with atypical abdominal imaging findings, to avoid unnecessary surgical intervention.

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A DIAGNOSTIC DILEMMA IN AN IMMUNOSUPPRESSED PATIENT WITH ADRENALITIS: A CASE REPORT

Jessica Tuan
Columbia University Medical Center, New York, NY, United States

A 66-year-old man from Dominican Republic (D.R.) (who traveled between U.S. & D.R., last 1 year ago) with nonischemic cardiomyopathy and multiple myeloma [received chemotherapy & autologous bone marrow transplant 10 years prior] became lost to follow-up and relapsed. Recently, he resumed Daratumumab/Pomalidomide-based chemotherapy. PET-CT scan showed increased uptake in a new 11mm left adrenal nodule, vertebrae, sclerotic rib & sacral lesions, thyroid, and mediastinal lymph node; stable right lung nodule with increased uptake & scattered small lung nodules. CT abdomen demonstrated 1.5x1.1cm left adrenal nodule. He underwent left adrenalectomy with histopathology demonstrating chronic adrenalitis with extensive granulomatous inflammation, focally necrotizing with yeast. GMS & PAS-d staining demonstrated numerous round-to-oval yeast (3-7μm) with occasional narrow-based budding. He was afebrile (T35.5°C) and hemodynamically stable with normal examination. Infectious workup (blood cultures, fungal antigens/antibodies, metagenomic blood sequencing) was unrevealing. Adrenal tissue fungal sequencing demonstrated *Histoplasma capsulatum*. His mild malaise improved with posaconazole. Initial concern for morphology per pathology suggested *Cryptococcus*, though this was less likely given Fontana-Masson staining was negative; and given some interpretation of possible broad-based budding also noted, the differential diagnosis was broadened to include Blastomycosis. Ultimately, the morphology demonstrating narrow-based budding helped reinforce the diagnosis of Histoplasmosis. Thus, it is important for clinicians to understand the microbiologic natural history of *Histoplasma* and also the novel diagnostic tools including fungal sequencing that can diagnose such an obscure clinical syndrome of *H. capsulatum* adrenalitis. This is especially true given initial infectious workup was unrevealing. Particularly, this diagnosis has significant clinical implications in immunosuppressed hosts with underlying history of relapsed malignancy and bone marrow transplant who require chemotherapy.

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PREDICTORS OF EMPIRIC ANTIBIOTIC USE AMONG HOSPITALIZED PATIENTS WITH ACUTE FEBRILE ILLNESS IN MOGADISHU, SOMALIA

Abyan Dimbil[1], Qadija Ali[2], Sophie Charlotte Schneitler[3]
[1]Jamhuriya University, Mogadishu, Somalia, [2]Jazeera University, Mogadishu, Somalia, [3]University of Saarland, Solingen, Germany

Acute febrile illness remains a major diagnostic challenge in Somalia, a data-poor region of critical public health concern. Previously, we analyzed hospitalized adult patients with febrile illness at a tertiary hospital in Mogadishu; etiologies were identified in only 24.2% of cases, with viral

infections representing the most common confirmed cause. This low diagnostic yield highlights the significant uncertainty clinicians face when managing febrile illness. In this secondary analysis, we evaluated patterns and predictors of empiric antibiotic use among the same cohort. Despite the predominance of unknown and viral etiologies, antibiotics were administered to 83.3% of patients. Predictors of antibiotic administration included leukocytosis (p<0.001), hypotension (p=0.01), thrombocytopenia (p=0.03), and elevated C-reactive protein (p=0.008), suggesting that clinicians rely on markers of systemic inflammation and clinical severity when initiating empiric therapy in the absence of definitive diagnostic results. These findings highlight the difficulty of antibiotic decision-making in patients with acute febrile illness in settings where etiology frequently remains unidentified. High rates of antibiotic use reflect ongoing diagnostic uncertainty and underscore the challenges of managing febrile illness in resource-limited settings. Strengthening diagnostic capacity and implementing antimicrobial stewardship strategies will be critical to guiding appropriate therapy. To our knowledge, this is the first study in Somalia examining predictors of empiric antibiotic use among hospitalized patients with acute febrile illness.

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IMPORTED MALARIA IN ITALY: RETROSPECTIVE STUDY OF MALARIA CASES DIAGNOSED AT THE I.R.C.C.S. SAN MATTEO HOSPITAL FOUNDATION (PAVIA, ITALY) FROM 2005 TO 2025

Gian Luca D'Alessandro[1], Roberta Maserati[2], Raffaella Lissandrin[3], Sofia Frattola[4], Raffaele Bruno[5], Enrico Brunetti[5]

[1]Emergency Department, ASST Melegnano Martesana, Vizzolo Predabissi, Italy, [2]Unit of Microbiology and Virology, IRCCS San Matteo Hospital Foundation, Pavia, Italy, [3]Unit of Infectious Diseases, IRCCS San Matteo Hospital Foundation, Pavia, Italy, [4]Department of Clinical-Surgical, Diagnostic and Pediatric Sciences, University of Pavia, Pavia, Italy, [5]University of Pavia, IRCCS San Matteo Hospital Foundation, Pavia, Italy

In Italy, malaria is a travel-related disease with low incidence. National epidemiological data on malaria are provided by the National Health System, but information about local dynamics such as the rate of missed diagnoses and the rate of patients first evaluated in primary or secondary facilities is unknown. The present study describes the epidemiological and clinical features of imported malaria cases diagnosed between January 2005 and August 2025 in our tertiary hospital, I.R.C.C.S. San Matteo Hospital Foundation, located in Pavia (northern Italy). We collected the records of all malaria blood films and RDT performed in the Parasitology Laboratory of our hospital and identified patients with a positive result for either of the two tests. A total of 206 cases were diagnosed with imported malaria in the study period (average 9.8 cases/year), 11.6% of which were pediatric patients. The majority of patients were natives of Africa (67.5%). The average age of the cohort is 36 years. We recorded a slight increase in the number of diagnoses comparing the period 2005-2014 (83 cases) with the period 2015-2024 (110 cases). An exception to this trend was the year 2020 (3 cases), when a drop in incidence reflected travel restrictions related to the COVID-19 pandemic. In 83.5% of cases, *P. falciparum* was the causative *Plasmodium* species. Severe malaria accounted for 16.5% of total cases. No deaths were reported. Malaria diagnosis was missed at first evaluation in 13.1% of cases. The site of first evaluation was the Emergency Department in the majority of cases (86.8%) and among these cases, 40.7% were seen in primary or secondary centers. Despite a mild increase recorded at a local level in the last 10 years, incidence remains low. A notable portion of patients are consistently first evaluated in primary/secondary care centers, which have less expertise in recognizing the disease. In 1 out of 8 patients, malaria diagnosis is missed at first evaluation, with prognostic and public health consequences. Continuous surveillance is critical, since the possibility for re-establishment of malaria in Italy is not null.

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REACHING THE UNREACHED: ADAPTIVE MASS DRUG ADMINISTRATION STRATEGIES TO OVERCOME LOW COVERAGE AND CROSS BORDER POPULATION MOBILITY IN ONCHOCERCIASIS HOTSPOTS IN MALI

Yaya Ibrahim Coulibaly[1], Diadje Tanapo[1], Moussa Sangare[1], Claudia Duguay[2], Mahamoud Mahamadou Koureichi[1], Sekou Oumarou Thera[1], Siaka Yamoussa Coulibaly[1], Salif Seriba Doumbia[1], Eve Gatawa[3], Anne Heggen[3], Abdoul Fatao Diabate[1], Yacouba Sangare[4], Alison Krentel[5]

[1]International Center of Excellence in Research, University of Sciences, Techniques and Technologies of Bamako, Bamako, Mali, [2]University of Ottawa, Ottawa, ON, Canada, [3]The End Fund, New York, NY, United States, [4]Ministere de la Sante Mali, Bamako, Mali, [5]University of Ottawa, Ottawa, Canada

Despite decades of ivermectin MDA, persistent transmission hotspots and suboptimal therapeutic coverage remain in parts of Mali. Of the 18 operational transmission zones (OTZs) in the country, only KA05 failed the countrywide onchocerciasis pre-stop survey in 2020. It is populated with vulnerable populations. The study aimed to identify drivers of low coverage and to codesign and pilot adaptive MDA delivery strategies in KA05. Three cross-sectional household surveys were conducted in KA05: one at baseline and two following subsequent yearly MDA. Six villages were selected based on onchocerciasis prevalence. Codesigned with stakeholders and informed by baseline findings, the strategies included targeted information, education, and communication materials; enhanced trainings for drug distributors; strengthened community engagement through stakeholder meetings; mobility adjusted distribution calendars; drug distribution in hamlets and mining sites, intensified supervision, and the use of WhatsApp groups and radio communication to improve awareness. Electronically captured data were analyzed using R software. A generalized linear mixed effects model was fit to identify determinants of MDA coverage. A total of 2,784 individuals were enrolled across the three survey rounds. Following implementation of adaptive strategies, MDA participation increased significantly, with individuals nearly twice as likely to participate during the second (aOR=1.95; 95% CI: 1.55,2.45) and third MDA rounds (aOR=1.95; 95% CI: 1.56,2.44) as compared to baseline. Older age and good knowledge of onchocerciasis were associated with higher participation, while traveling during the MDA period significantly reduced uptake (aOR=0.54; 95% CI: 0.45,0.66). This strategy improved MDA participation. While older age and better knowledge of onchocerciasis were associated with higher uptake, travel during the MDA period remained a significant barrier. Scaling these adaptive strategies will be critical to reaching mobile populations and accelerating progress toward WHO onchocerciasis elimination.

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RESULTS OF A PROFICIENCY TESTING SCHEME FOR MOLECULAR TESTING OF WEST NILE VIRUS

Damien Garcin[1], Elaine McCulloch[2], Tyler Carney[3], Oliver Donoso Mantke[2]

[1]altona Diagnostics, Hamburg, Germany, [2]QCMD, Glasgow, United Kingdom, [3]altona Diagnostics USA Inc, Plain City, OH, United States

Molecular techniques in West Nile Virus (WNV) RNA testing are standard in Europe for blood and organ donation screening from subjects from endemic WNV areas, and in the identification of viral genomes in different sample matrices from patients presenting a clinical picture typical of WNV infection. As diagnostics in this area evolves and increasing number of commercial assays have come to market, an understanding of the limitations of some assays is important to assess when implementing into clinical practise. To allow laboratories to assess their proficiency in the detection of WNV and differentiation from other flaviviruses, proficiency testing (PT) schemes are an invaluable tool. WNV RNA PT samples over 5 consecutive years were analysed. Non-WNV Flaviviruses like Dengue Virus Type 1(DENV-1), Japanese Encephalitis Virus (JEV), Yellow fever Virus (YFV),

Zika Virus (ZIKV), and/or Usutu Virus (USUV) samples were included in the panels. Out of in total 50 samples, 38 samples were defined WNV samples with different viral loads, 7 were specificity samples (Non-WNV Flavivirus) and 5 samples were true negatives. Specificity non-WNV Flavivirus samples were correctly tested as negative for WNV by 90% of the in-house PCR assays and commercial assays. The USUV samples were correctly tested negative with the WNV specific in-house PCR assays, whereas some of the commercially available assays gave false positive results for WNV RNA. Cross-reactivity was observed for some WNV molecular tests. The clinical relevance of non-detected or as WNV-positive detected USUV infection in humans and the risk of USUV to blood safety are unknown however needs to be carefully considered.

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CLINICAL AND HEMATOLOGICAL CORRELATES OF DENGUE-ASSOCIATED CHOLECYSTITIS: A HOSPITAL-BASED CROSS-SECTIONAL STUDY IN BANGLADESH

Mehnaz Kamal[1], Shaila Rahman[2], Amit Wazib[3]

[1]Tulane University, New Orleans, LA, United States, [2]Shaheed Monsur Ali Medical College Hospital, Dhaka, Bangladesh, [3]Bangladesh Medical College, Dhaka, Bangladesh

Bangladesh experiences frequent dengue outbreaks in addition to its endemic situation, and acute acalculous cholecystitis is increasingly recognized as part of expanded dengue syndrome. This cross-sectional study included 203 confirmed dengue patients admitted to the Department of Medicine at a medical college hospital in Dhaka, Bangladesh, from July to October 2023, to evaluate the association between dengue-associated cholecystitis and demographic factors, comorbidities, hematological parameters, complications, blood product use, and duration of hospital stay. Dengue-associated cholecystitis occurred in 10.8% of patients, and all cases were acalculous. Significant associations were observed with diabetes ($p=0.03$), higher hematocrit ($p=0.001$), and lower platelet count ($p<0.001$). Hematocrit ≥43% and platelet count ≤45,000/cumm were identified as useful predictors. Patients with dengue-associated cholecystitis had a significantly higher frequency of dengue hemorrhagic fever and dengue shock syndrome ($p<0.001$ for both), required more blood products ($p<0.001$), and had longer hospital stay ($p=0.01$). All patients with dengue-associated cholecystitis recovered with conservative management. Dengue-associated cholecystitis was associated with increased disease severity and greater resource utilization. Larger prospective multicenter studies are recommended to evaluate these findings further.

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OBTAINING HUMAN SAMPLES IN AUSTERE ENVIRONMENTS FOR RESEARCH: CHALLENGES AND LESSONS LEARNED

Rita Corrales[1], Mark Cashin[2], Jen Zimmerer[2], Wendy Boone[2], Joseph Sgherza[2], Julius Julian Lutwama[3], Mark G. Kortepeter[4]

[1]Gorgas Memorial Institute, Dept of Research in Virology and Biotechnology, Panama, Panama, [2]Integrum Scientific LLC, Beaufort, NC, United States, [3]Uganda Virus Research Institute, Entebbe, Uganda, Uganda, [4]Uniformed Services University of the Health Sciences, Bethesda, MD, United States

Understanding the immune response to infectious diseases in order to develop effective countermeasures relies heavily on obtaining human samples for immunologic study from acutely or previously infected volunteers. Obtaining such samples may present a challenge, even for infections that are endemic in developed settings in the "Global North." The challenge becomes more acute in less accessible regions of the "Global South," where infections may surface intermittently or in logistically difficult areas to access. This may be compounded by limited access to power and transportation, weather and force majeure challenges, limited scientific or regulatory infrastructure, unfavorable diplomatic relations with a specific country where a disease is endemic or epidemic, armed conflict, language barriers, and lack of an easily identifiable in-country partner. However, sometimes such challenging settings are the only areas to obtain valuable human samples for study. A successful partnership to obtain and analyze valuable human samples relies on several important aspects. Integrum Scientifics has worked successfully with research partners in five countries in the Global South to obtain serum, plasma, and PBMCs in support of diagnostic, therapeutic, and vaccine development on 10 different infections for mutual benefit. Herein we share some challenges we have encountered and overcome related to partnership development, regulatory/ethical aspects, force majeure considerations, logistics of sample collection, processing, and cold chain storage and transportation. We then provide lessons learned and recommendations for proactive risk mitigation to plan for success.

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AN ISLAND OF ISOLATION IN AN ENDEMIC SEA: INSIGHTS FROM A DOG BITE IN THE ANDAMAN ISLANDS

Apoorva Pandharpurkar

Insight Hospital and Medical Center Chicago, Chicago, IL, United States

One of the oldest known communicable diseases, rabies with its painful and uniformly fatal nature has haunted civilisations for millennia, becoming the subject of superstition and folklore. With India bearing the highest global burden of rabies and accounting for nearly one-third of worldwide deaths, prevention remains a major public health priority. As a dog lover who spent her childhood caring for strays, it was never lost on me how these otherwise friendly animals can become dangerous once infected, serving as the primary reservoir and transmitter of rabies to humans in India. The Government of India addresses this burden through the National Rabies Control Program, a multi-level public health initiative aimed at reducing transmission through coordinated animal and human health strategies. Its efforts span animal control strategies such as trap neuter release and culling of infected animals and vaccinating animals free of cost alongside a robust post exposure prophylaxis program. This includes free vaccination at public hospitals with a 5 dose schedule over 28 days, as well as rabies immunoglobulin administration for Category III exposures. As part of my medical training in India, I was trained in triaging exposures and administering PEP. Little did I know that I would find myself on the other side of the doctor patient interface. During a vacation to the Andaman and Nicobar Islands in my intern year, an unexpected dog bite led to a deeper exploration into rabies epidemiology. On presenting to a local hospital, I was informed that the islands have historically remained rabies free, an intriguing and impressive achievement given their location amidst highly endemic regions including mainland India, Bangladesh, and Southeast Asia. This experience highlights a unique epidemiologic paradox. The sustained rabies free status of the Andaman Islands suggests the effectiveness of geographic isolation, strict animal importation controls, and coordinated public health measures. Understanding how such regions maintain freedom from rabies may offer valuable insights into scalable strategies for elimination in endemic settings.

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PROFILE AND OUTCOME OF REFERRED PEDIATRIC PATIENTS AT THE UNIVERSITY OF KINSHASA HOSPITAL IN THE DEMOCRATIC REPUBLIC OF THE CONGO

Nadège Buroko NSIMIRE[1], Diane-Véronique Kondani Apeangangn[2], Mireille Ngale Amba[2], Celestin Nsibu Ndosimao[3], Nsengi Yumva Ntamabyaliro[4]

[1]University of Kinshasa, Kinshasa, Democratic Republic of the Congo, [2]Department of Pharmacology, University of Kinshasa, Kinshasa, Democratic Republic of the Congo, [3]Department of Pediatrics, University of Kinshasa, Kinshasa, Democratic Republic of the Congo, [4]Department of Pharmacology, University of Kinshasa, Kinshasa, Republic of the Congo

Child mortality remains high in low-resource countries like the Democratic Republic of the Congo (DRC), largely due to preventable conditions requiring timely and appropriate care. Referral of ill children to better-equipped hospitals is essential to improve outcomes. However, referral systems are often suboptimal, leading to increased risk of adverse outcomes. Understanding the most frequent reasons for referral is critical to anticipate resource needs and optimize preparedness for appropriate

management. In the DRC, data on the characteristics and outcomes of referred pediatric patients are scarce. This study aimed to assess the clinical characteristics and therapeutic outcomes of children referred to the pediatric department at the University of Kinshasa Hospital (UKH), the highest-level referral center in the DRC. This retrospective observational study was conducted in the pediatric department of UKH, reviewing medical records of children transferred between January 1 and December 31, 2024. Data were collected electronically via Kobotoolbox from chart reviews in emergency and intensive care units. All eligible, complete records of transferred patients were included. Of the 143 children referred during the study period, (51% male, 62.2% aged 6–59 months), most were referred from health centers (65.7%). Transfers were mainly for respiratory distress (32.2%) and anemia (15%). Severe malaria was the leading diagnosis (24.5%), followed by meningitis/meningoencephalitis (15.4%). Outcomes: 41.2% recovered, 36.4% stabilized, with a 22.4% fatality rate; there were no deaths among severe malaria cases. Infectious diseases, particularly malaria, respiratory infections, and meningitis, are common among transferred pediatric patients. Mortality is high in children referred with respiratory and neurological symptoms, though severe malaria management is very effective. Improved equipment for critical cases, especially respiratory and neurological emergencies, is essential to lower mortality rates.

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RISK PERCEPTION AND PSYCHOLOGICAL DISTRESS AMONG HEALTHCARE WORKERS DURING THE FIRST COVID-19 WAVE IN CUSCO, PERU

Andy J. Alvarado-Yepez[1], Andres G. Lescano[1], Fredy S. Monge-Rodriguez[2]

[1]Emerge, Emerging Diseases and Climate Change Research Unit, School of Public Health and Administration, Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Universidad Peruana Cayetano Heredia, Lima, Peru

Healthcare workers faced intense psychological distress during the first wave of the COVID-19 pandemic, particularly in settings characterized by health system collapse and high mortality. Risk perception is a key determinant of psychological responses, but most studies only use unidimensional measures and do not account for complex contextual factors. This study evaluated the association between multidimensional COVID-19 risk perception and psychological distress among healthcare workers in Cusco, Peru during the first pandemic wave. We studied COVID-19 perceptions and mental health among frontline healthcare workers in Cusco, Peru, late in the first wave. A cross-sectional virtual survey assessed risk perception using a six-item multidimensional index, and psychological distress was measured with the SA-45 Global Severity Index. Associations were evaluated using Poisson regression models with robust variance, adjusting for confounders identified through a directed acyclic graph. Among 241 healthcare workers, the prevalence of psychological distress was 42.7%, and 75.0% reported high levels of risk perception. Higher levels of risk perception were associated with a borderline greater prevalence of psychological distress (adjusted prevalence ratio [aPR]=1.44, 95% CI 0.96-2.16), with similar estimates observed across intermediate risk perception levels. Greater confidence in COVID-19 information was significantly associated with lower psychological distress (aPR=0.84, 95% CI 0.76-0.95). Post hoc analyses showed that risk perception was consistently associated with specific symptom domains, including anxiety, phobia, somatization, and obsessive-compulsive symptoms. Our findings suggest that, in a context of high system overload and prolonged exposure to the pandemic, psychological distress among healthcare workers may have been driven more by immediate occupational and contextual factors than by perceived risk alone. Improving access to reliable information and addressing structural stressors may help mitigate psychological distress in future health emergencies.

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KNOWLEDGE, PRACTICES AND ATTITUDE OF RESIDENTS TOWARDS BURULI ULCER IN JASIKAN MUNICIPALITY: AN ETHNOGRAPHIC STUDY

Joseph Alale Aweeya

Navrongo Health Research Centre, Navrongo, Ghana

Buruli ulcer, caused by *Mycobacterium ulcerans*, is a neglected tropical disease characterized by progressive skin and soft tissue destruction. Despite ongoing control efforts, disease transmission remains poorly understood, and delays in care-seeking continue to contribute to morbidity and disability in endemic settings. Socio-cultural perceptions and community-level behaviors play a critical role in shaping health-seeking pathways and disease outcomes. This study explored community knowledge, practices, and socio-cultural attitudes towards Buruli ulcer in Jasikan Municipality, Ghana, to identify behavioral and contextual factors influencing treatment-seeking and disease management. An ethnographic qualitative study was conducted among 20 purposively selected participants in Jasikan Municipality. Data were collected through in-depth interviews using a semi-structured guide and analyzed using thematic analysis. Ethical approval was obtained from the appropriate institutional review board. Participants demonstrated variable and often incomplete knowledge of Buruli ulcer, with persistent misconceptions regarding its causes and transmission. Health-seeking behavior was pluralistic, involving biomedical facilities, traditional healers, prayer camps, and self-medication. Stigma and social exclusion of affected individuals were commonly reported and contributed to delayed presentation and treatment discontinuation. Inadequate wound care practices and reliance on non-medical treatment pathways further hindered effective disease management. Socio-cultural beliefs, stigma, and fragmented healthcare pathways significantly influence Buruli ulcer management in endemic communities. Interventions should extend beyond conventional health education to incorporate culturally responsive, community-engaged strategies that reduce stigma, promote early care-seeking, and strengthen integration between formal and informal healthcare systems to improve NTD control outcomes.

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PRETERM CONGENITAL *PLASMODIUM VIVAX* MALARIA IN AN ASYMPTOMATIC MOTHER: A CASE REPORT FROM AN ENDEMIC AREA IN THAILAND

Ajjima Thussachan[1], Wanlapa Roobsoong[2]

[1]Mahidol University, Ratchathewi, Thailand, [2]Ratchathewi, Thailand

We report a rare case in Thailand of congenital *Plasmodium vivax* infection in a 20-day-old preterm neonate, 33 weeks' gestation as assessed by Ballard score, born in a malaria endemic region of Thailand. The infant was delivered in a hospital and remained in the neonatal intensive care unit (NICU) from birth, with no opportunity for postnatal mosquito exposure. At 20 days of life, the infant developed a fever and underwent neonatal sepsis evaluation. An automated complete blood count analyzer flagged infected red blood cells (iRBCs), and subsequent thin blood smear demonstrated few *P. vivax* trophozoites in ring and amoeboid forms. Quantitative RCR (qPCR) confirmed *P. vivax* infection in both the mother and the infant. Notably, the mother was asymptomatic, with no reported history of fever or malaria-related symptoms during pregnancy or after giving birth. Serological testing supported recent maternal exposure to malaria infection. The infant was treated with chloroquine alone, as congenital malaria does not involve a hypnozoite stage. The mother received chloroquine followed by primaquine after confirmation of normal glucose-6-phosphate dehydrogenase (G6PD) status. This case provides strong evidence of vertical transmission of *P. vivax* from an asymptomatic mother, supported by molecular detection in both mother and infant and the absence of postnatal exposure. This report highlights the potential role of asymptomatic maternal infection as an underrecognized source of congenital malaria in an endemic setting, with the elimination plan of malaria in process. It underscores the need for improved screening strategies during pregnancy, particularly in endemic

areas, and for increased clinical awareness of congenital malaria in neonates presenting with non-specific or sepsis-like symptoms, even in the absence of maternal illness.

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CLINICAL FEATURES AND OUTCOMES OF *STREPTOCOCCUS SUIS* MENINGITIS IN A LONGITUDINAL STUDY OF SEPSIS IN TAKEO PROVINCE, CAMBODIA

Alyse Wheelock[1], Yasith Ros[2], Monira Meas[2], Sengly Chor[2], Sengheng Chor[2], Vannsay Yin[2], Patrick J. Blair[3], Te Vantha[4]

[1]The Henry M Jackson Foundation for the Advancement of Military Medicine, Bethesda, MA, United States, [2]Lucerent Clinical Solutions Limited, Takeo Province, Cambodia, [3]The Henry M Jackson Foundation for the Advancement of Military Medicine, Bethesda, MD, United States, [4]Takeo Provincial Referral Hospital, Takeo Province, Cambodia

Streptococcus suis infection is a zoonosis associated with contaminated pork intake in Southeast Asia. Invasive infection causes meningitis and sepsis. This case series describes presentations and longitudinal outcomes among 7 individuals with *S. suis* meningitis in Takéo Province, Cambodia. In an on-going longitudinal study of sepsis in Takéo Province, Cambodia (N=749), 50 participants have been determined to have suspected, probable, or confirmed meningoencephalitis by two external adjudicators; a causative pathogen was identified in 15 participants. Of these, 7 participants were found to have microbiologically-confirmed *S. suis* meningitis. Their ages ranged from 33-66 years (median 43), 6/7 were male, and 3/7 reported working in abattoirs; they were enrolled between 2015-2018. All 7 participants presented with acute onset headache and fever; 6/7 participants additionally reported both visual blurring and hearing difficulties on admission. Blood cultures were positive for *S. suis* in 4/7 participants. Median peripheral white blood cell count at enrollment was 20.5×10^9/L (range 8.5 - 23.4× 10^9/L), falling to 14.6×10^9/L (range 9-22.4 × 10^9/L) at 24 hours. Median cerebrospinal fluid white blood cell count was 2,114 cells/mm³ (range 84 - 10,824 cells/mm). Treatment included systemic steroids for 5 participants. Median length of stay was 9 days (range 1-19 days) and all participants were discharged alive, though headache persisted for 4, confusion for 3, and hearing difficulties for 2 participants at time discharge. One participant was lost to follow-up; all other participants were alive at 28-day followup, with 6/6 reporting headache then. Within a longitudinal cohort of sepsis in Takéo Province, Cambodia, *S. suis* was the most common confirmed pathogenic cause of meningoencephalitis. While there was no short-term mortality among participants with *S. suis* meningitis, audio and visual symptoms were frequently reported and all participants had persistent headache at 28 days. No recent cases have been detected within this actively enrolling study, suggesting a possible etiologic shift in meningoencephalitis in Takéo Province.

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EVALUATION OF ANTIBODY-DEPENDENT CELLULAR CYTOTOXICITY (ADCC) SYSTEM IN NON-HUMAN PRIMATE MODEL TO STUDY SHIV INFECTION

Sandra Steffany Morales Ruiz, Natsuko Shimazaki, Otsuki Mai, Hisatoshi Shida, Tomoyuki Miura

Primate model laboratory, Institute for Life and Medical Sciences, Kyoto University, Kyoto, Japan

Non-neutralizing antibodies (nnAb) involved in antibody-dependent cellular cytotoxicity (ADCC) mechanism have been associated with reduction of HIV infection risk during RV-144 HIV trial. ADCC has been widely studied in HIV infection; however, to date, there is no consensus method for ADCC evaluation, hindering its study in non-human primate (NHP) models. This includes limitations of current methods such as inter-species cross-reactivity between monkey Fc and human Fc receptor, and overestimation caused by non-specific killing of uninfected bystander cells. The goal was to establish a fully species-matched, flow cytometry ADCC evaluation system for NHP models using SHIV-infected target cells, rhesus macaque PBMCs as effector cells and antisera collected at early (6 weeks post infection) and late stage (50 and 52 wpi) of infection from SHIV-infected rhesus macaques as antibody resource. Additionally, SIV anti-p27 Fab was included for intracellular detection of productively infected cells to avoid ADCC activity overestimation. For antibody binding evaluation, SHIV-infected cells were evaluated by flow cytometry using 1000-folded diluted antisera collected at early and late stage of infection, characterized as negative and positive for neutralizing activity, respectively. Surprisingly, all antisera including the neutralizing negative antisera (early stage) recognized SHIV-infected cells. Then, each antiserum was used for ADCC evaluation. All antisera mediated ADCC activity. Notably, ADCC activity mediated by early-stage antisera was either similar or higher than late-stage antisera. These findings suggest that despite early-stage antisera was negative for neutralizing activity, they mediated ADCC activity in SHIV-infected rhesus macaques, highlighting that ADCC activity does not necessarily correlate with neutralizing activity. Therefore, the presence of nnAb at early stage of infection could contribute to mount an early immune response that allows the elimination of infected cells. Finally, this evaluation system allowed to measure ADCC activity in NHP models.

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BURDEN AND CHALLENGES IN DIAGNOSIS AND TREATMENT OF HISTOPLASMOSIS AMONG ADVANCED HIV DISEASE PATIENTS IN UGANDA

Viola Kasone[1], Ritah Namusoosa[2], Judith Ainemukama Kyokushaba[2]

[1]Viola Kasone, kampala, Uganda, [2]Kampala, Uganda

Data on the true burden of histoplasmosis in Uganda are limited, largely due to the scarcity of epidemiological studies. We aimed to describe the nationwide burden of histoplasmosis among persons with advanced HIV disease (AHD), highlighting the major challenges in diagnosis and treatment under routine HIV care. This was a prospective longitudinal study conducted at nine health facilities in Uganda. Persons with AHD were included and followed up for two years. Urine samples were sent to the Central Public Health Laboratories and tested using the *Histoplasma* galactomannan enzyme immunoassay in batches. Histoplasmosis was defined using standard criteria.We enrolled 332 participants with AHD, and 66.6% were newly diagnosed with HIV. Over half were female (51.5%), with a median age of 35 years (Interquartile range - IQR=29-42) for all participants. The median CD4 T-cell count was 73 cells/μL (IQR=30.5-126.5). Eight (2.4%) participants were diagnosed with probable histoplasmosis. None of the cases received the recommended antifungals because they were not available in the country. After two years, five cases were still alive, two were lost to follow-up, and one died. Major challenges included missed diagnosis, low index of clinical suspicion, overlap of symptoms with those of pulmonary TB, high cost, and limited access to antifungals such as voriconazole, itraconazole, radiology and laboratory investigations, and lack of knowledge about treatment guidelines.**Conclusions:**Histoplasmosis occurs at a low frequency among persons with AHD in Uganda. Numerous challenges hinder a definite diagnosis and treatment. However, despite these challenges, survival among persons with AHD in Uganda who have untreated histoplasmosis is relatively good.

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IMPACT OF HEPATITIS B VIRUS AND HEPATITIS C VIRUS COINFECTION ON HUMAN IMMUNODEFICIENCY VIRUS TREATMENT RESPONSE AMONG PATIENTS RECEIVING ANTIRETROVIRAL THERAPY IN THE BONO REGION OF GHANA

Agyemang Duah[1], Patrick W. Narkwa[1], Maxwell O. Peprah[2], **Seth A. Domfeh**[1], Mohamed Mutocheluh[1]

[1]Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, [2]Presbyterian Nursing and Midwifery Training College, Dormaa Ahenkro, Ghana

Hepatitis B virus (HBV) and hepatitis C virus (HCV) coinfections remain crucial comorbidities among individuals living with human immunodeficiency virus (HIV), particularly in subSaharan Africa, where all three viral infections

are endemic. Coinfection can accelerate liver disease progression and adversely affect antiretroviral therapy (ART) outcomes. Therefore, this study evaluated the impact of HBV and HCV infection on the prognosis of HIV patients on ART. One hundred adults initiating firstline ART at the Dormaa Presbyterian Hospital in the Bono Region of Ghana were enrolled in this prospective cohort study. Baseline sociodemographic and clinical data were collected through questionnaires, and venous blood samples were analysed for HBsAg, antiHCV IgM, haematological indices, liver transaminases, and HIV load. Patients were followed for six months after ART initiation. The overall prevalence of viral hepatitis was 22%, comprising HBV (11%), HCV (9%), and HBV-HCV coinfection (2%). No significant changes in haematological indices were observed over the six months, except in the HIV-HCV co-infected patients, whose platelet count significantly declined, and the HIV-HBV and HIV-HBV-HCV co-infected patients, whose WBC count significantly increased over time. The liver transaminases (AST and ALT) increased significantly in HBV-HIV and HCV-HIV co-infected groups, indicating heightened hepatic stress following ART initiation. Although the overall HIV load decreased significantly after six months, coinfected patients consistently exhibited higher viral loads than HIV monoinfected patients throughout the followup. These findings indicate that HBV and/or HCV coinfection influences ART response and contributes to liver function abnormalities during treatment. Consequently, routine screening for HBV and HCV before ART initiation is recommended to optimise clinical management. Larger multisite studies are warranted to inform national policies on integrated HIV-viral hepatitis care in Ghana.

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EFFECT OF FACILITY TYPE ON RETENTION AMONG ADULTS INITIATING ANTIRETROVIRAL THERAPY, 2021 TO 2024

Keith L. Gray[1], Sam J. Gebeh, II[1], Jonathan Flomo[2], Claudius B. Paye[2]

[1]Boston University School of Public Health, Boston, MA, United States, [2]Ministry of Health, Monrovia, Liberia

Many sub-Saharan countries have scaled Differentiated Service Delivery (DSD) models to improve HIV outcomes, yet Liberia's national response remains largely facility based. Facility-level differences may influence retention and treatment success. We emulated a target trial to estimate the causal effect of facility tier on loss-to-follow-up (LTFU) at 12 and 24 months and viral suppression among adults initiating antiretroviral therapy (ART). Inverse probability of treatment weighting from a mixed-effects logistic model with county-level random intercepts was used to compare patients initiating care in health centers versus hospitals. LTFU was defined as missing a scheduled visit by >28 days. Death was treated as a competing risk, using Fine and Gray model with a doubly robust approach. Absolute risk differences (RD) in the cumulative incidence were estimated via marginal standardization with bootstrapped 95% confidence intervals. Viral suppression (<1000 copies/ml[3]) among individuals with viral load measurements was evaluated using weighted generalized estimating equations with an identity link and clustering at the county level. 7318 individuals contributed to 5704 trial-participants, with 35.1% (n=2000) initiating care at health centers. The median follow-up was 24 months. The cumulative incidence of LTFU was 33.0% (30.7-35.2) at 12 months and 39.7% (37.0-42.3) at 24 months. LTFU was consistently lower in health centers. At 12 months, the adjusted cumulative incidence was 23.9% (20.7 -27.1) in health centers versus 35.4% (31.9-38.8) in hospitals (RD: -11.4 percentage points; -15.3 to -7.6). At 24 months, estimates were 29.3% (25.7-32.9) and 42.5% (38.6-46.3) (RD: -13.2 percentage points; -17.2 to -9.2). Among those with viral load measurements (n=1903), 74.5% were virally suppressed. Adjusted suppression was 69.0% (62 - 74) in health centers and 74% (64 - 84) in hospitals (RD: -5.1 percentage points; -12.8 to 2.5), with no marked difference. ART initiation at health centers reduced the risk of LTFU, supporting decentralized care. Policies should prioritize addressing structural barriers to retention in hospitals.

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CRYPTOCOCCAL ANTIGENEMIA IS ASSOCIATED WITH HIGH MORTALITY IN ADAMA, ETHIOPIA: IDENTIFYING GAPS IN HIV CARE

Semele Zewde[1], Tafese Beyene Tufa[2], Abera Balcha Bulti[3], Asiya Jeylan[4], Girma Mulisa[4], Godana Jarso[4], Radha Rajasingham[5], Anteneh Zewde[6], Caleb Skipper[6]

[1]University of Kentucky College of Medicine, Lexington, KY, United States, [2]Asella, Ethiopia, [3]Geisinger Clinic, Scranton, PA, United States, [4]Adama Hospital Medical College, Adama, Ethiopia, [5]Minneapolis, MN, United States, [6]University of Minnesota, Minneapolis, MN, United States

Individuals with advanced HIV disease, defined as CD4 count <200 cells/μL or presenting with WHO Stage 3/4 AIDS-defining illness, are increasingly susceptible to opportunistic infections—including cryptococcal meningitis. The World Health Organization recommends a package of care for all people with advanced HIV disease, including cryptococcal antigen (CrAg) testing. However, consistent implementation in low resource settings like Ethiopia face barriers such as limited diagnostic capacity, medicine stock outs, and unstructured referral pathways. We conducted an observational screening study among persons with HIV and CD4 count <200 cells/μL at two clinical sites in central Ethiopia to determine the prevalence of cryptococcal antigenemia and clinical outcomes at six month. CD4 testing was performed with Visitect semi-quantitative CD4 assay (Omega Diagnostics; Alva, Scotland, UK) and serum CrAg testing by CrAg lateral flow assay (IMMY; Norman, OK, USA). Participants were screened for meningitis symptoms and referred to local care for clinical management. Vital status was determined at six months. Overall, 273 people with advanced HIV disease were enrolled, and 11.2% (31/273) were CrAg+. Headaches were reported in 19% of CrAg+ participants vs 6.8% of CrAg-negative participants; while 77% of those CrAg+ vs 91% CrAg-negative reported no meningitis symptoms (p=0.02). At six months, 32% (10/31) of CrAg+ persons were dead or lost to follow up, where as 16% (39/240) were dead or lost to follow up among CrAg-negative individuals (risk ratio = 1.99; 95% CI, 1.11-3.57; chi-sq P=0.03). CrAg+ persons at our sites in Ethiopia had slightly lower 6-month survival rates (67.7%) than similar cohorts in Uganda (75.2%) and Malawi (71.4%). Overall, CrAg+ individuals had 2-fold increased risk for death or lost to follow up than CrAg-negative individuals. Further attention to advanced HIV disease screening programs in Ethiopia are warranted, including ongoing research to identify gaps in diagnosis and management of AIDS-related infections.

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PANCYTOPENIA SECONDARY TO CO-INFECTION: MIXED MALARIA - DENGUE WITH WARNING SIGNS

Marian Marcela Muskus Montiel[1], Aura Cristina Hoyos Pastrana[2], Maria Fernanda Yasnot[3]

[1]University of Cordoba, Monteria, Colombia, [2]University of Cordoba, Montería, Colombia, [3]Monteria, Colombia

A 25-year-old female patient with no relevant medical history, with a history of travel to rural area endemic for vector-borne diseases. She presented with a 15-day history of unquantified fever, chills, and myalgia. On admission, her vital signs were BP 100/70 mmHg, HR 100 bpm, T° 38.5 °C, RR 16 bpm, and O_2 saturation 98%. Physical examination revealed abdominal pain on deep palpation. Laboratory tests reported hemoglobin 8.2 g/dL, white blood cells 4.38 × 10³/μL, and platelets 50,000/μL. In the thick smear, parasitic forms consistent with *Plasmodium vivax* were observed. However, molecular detection using conventional PCR confirmed a mixed infection with *P. falciparum* and *P. vivax*. IgG and IgM antibody tests for dengue were positive. Treatment with chloroquine and primaquine was initiated; however, given the progressive deterioration of hematological parameters, it was decided to transfer the patient to the intensive care unit. The antimalarial treatment was switched to an intravenous regimen with artesunate, and supportive care with intravenous fluids was initiated. After four days of treatment, the patient showed clinical improvement and hematological recovery (Hb 10 g/dL, white blood cells 6.0 x 10³/

µL, platelets 170,000/µL). Co-infection with malaria and dengue poses a diagnostic challenge in endemic areas due to the initial clinical overlap characterized by acute febrile syndrome, headache, and muscle pain. While *P. vivax* and *P. falciparum* malaria is classically associated with anemia secondary to intravascular and extravascular hemolysis, dyserythropoiesis, and splenic sequestration, dengue is characterized by thrombocytopenia, hemoconcentration and immune-mediated alterations that promote capillary leakage. In the case presented, the patient developed moderate anemia and severe thrombocytopenia with progressive deterioration that required treatment in the intensive care unit. The favorable outcome following intravenous antimalarial treatment and hemodynamic support underscores the importance of early recognition of co-infection.

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CUTANEOUS LEISHMANIASIS IN TIGRAY, ETHIOPIA: CLINICAL PATTERNS, ENVIRONMENTAL DRIVERS AND PUBLIC HEALTH IMPLICATIONS

Shewaye Belay Tessema

Mekelle University, College of Health Sciences, Mekelle, Ethiopia

Cutaneous leishmaniasis (CL) is widely distributed in the highlands of Tigray, Northern Ethiopia. However, little is known about the actual epidemiology of the disease in Tigray. Therefore, this study aimed to document the epidemiology, risk factors and public health implications of CL in Tigray, Ethiopia. Between March and May 2022, a cross-sectional household survey was conducted in seven districts of Tigray, using multistage sampling. Participants were clinically examined for scars and/or active lesions and samples taken from active lesions were investigated for parasite amastigotes by microscopy. Data on socio-demographic characteristics and environmental determinants were documented using semi-structured questionnaires. Bivariate and multivariate logistic regressions were used to identify risk factors associated with CL infections. A total of 3,817 individuals (48.2% males and 51.8% females) in 927 households were screened. A total of 484 individuals showed clinical evidence of CL (13.7% males and 11.5% females). The overall prevalence was 12.7% (4.5% active lesions, 8.2% scars). Active cases were predominantly localized (LCL) (85%) but a substantial number of mucocutaneous (MCL) cases (11%) and diffused (DCL) (4%) types were also identified. The highest rates of active lesions (5.2%) were in children aged 1-14 years. Most lesions (69%) occurred on face, and 51.4% of cases had ≥ 2 lesions. Cases clustered in highland zones (70.2% at 2,300-3,200 m altitude; 93% at 2,000-3,000 m). Significant host risk factors included age, outdoor sleeping and poor housing. Moreover, proximity to hyraxes, bats, caves, or animal burrows were among significant environmental risk factors ($p < 0.05$). Cutaneous leishmaniasis is an underprioritized yet a serious public health challenge in Tigray, driven by environmental and behavioral factors. Several forms of the disease (LCL, MCL and DCL) are prevalent, with children being the most affected. Targeted interventions for high-risk groups, enhanced surveillance and mapping, vector and reservoir control measures, community awareness and healthcare capacity are highly recommended.

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DIFFERENTIAL IMMUNE CELL DYNAMICS ACROSS CENTRAL AND PERIPHERAL IMMUNE COMPARTMENTS IN ACUTE AND CHRONIC CHAGAS DISEASE

Jung Sun Cho[1], Supriya Kumar[2], Erica Silberstein[3], Alain Debrabant[1]

[1]FDA/CBER/OBRR, Silver Spring, MD, United States, [2]Silver Spring, MD, United States, [3]CBER/FDA, Silver Spring, MD, United States

Chagas disease, caused by the blood borne parasite *Trypanosoma cruzi*, affects 6-8 million people worldwide and progresses from acute to chronic phases with potentially life-threatening cardiac and gastrointestinal complications. While immune responses are critical for parasite control and disease pathology, most studies examine single immune compartments, leaving uncertain how primary and secondary lymphoid organs are coordinately remodeled during disease progression. We performed immune cell profiling of bone marrow, thymus, spleen, and peripheral blood collected from the same C57BL/6 mice infected with *T. cruzi* at acute (1 month) and chronic (3 months) time points. Using spectral flow cytometry, we quantified $CD3^+$ T cells and their subsets including $CD4^+$, $CD8^+$, and double-negative T cells across all four compartments. We observed striking organ-specific and stage-specific immune remodeling. Primary lymphoid organs showed distinct dynamics with bone marrow $CD8^+$ T cells increasing five-fold during acute infection and remaining elevated chronically, while thymus showed only modest changes. In contrast, secondary lymphoid organs including spleen and peripheral blood exhibited remarkably parallel responses with total T cells expanding significantly during acute infection, driven primarily by $CD8^+$ T cell expansion, then contracting but remaining significantly elevated chronically. Double-negative T cells displayed divergent patterns with sustained elevation specifically in peripheral blood while remaining stable in other compartments. Our multi-organ analysis reveals compartmentalized immune remodeling during *T. cruzi* infection, with coordinated responses in secondary lymphoid organs contrasting with distinct dynamics in primary lymphoid organs. These findings underscore the importance of multi-compartment analysis for understanding Chagas disease immunity and may inform biomarker development and therapeutic strategies.

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ACTIVIN A EXPRESSION ASSOCIATES WITH HOST RESISTANCE IN A MURINE MODEL OF CUTANEOUS LEISHMANIASIS

Gina Slabber[1], Rebeng Maine[1], Natalie Nieuwenhuizen[2], Ramona Hurdayal[1]

[1]Department of Molecular & Cell Biology, University of Cape Town, Cape Town, South Africa, [2]Institute for Hygiene and Microbiology, Julius Maximilians University of Würzburg, Würzburg, Germany

Cutaneous leishmaniasis (CL) remains a global health burden with no licensed vaccine and current treatments limited by toxicity. Host-directed therapies have emerged as a promising strategy, requiring identification of novel immune mediators that govern disease outcome. Activins are pleiotropic members of the transforming growth factor-*B* (TGF-*B*) superfamily, distinguished by the dimerization of inhibin (Inh) *B*A and/or Inh*B*B subunits. Although they are established regulators of innate and adaptive immunity in infectious diseases, their role in CL remains entirely uncharacterized. Given their influence on macrophage polarization and T helper cell differentiation, key determinants of resistance and susceptibility in CL, this study provides the first characterization of activin expression after *Leishmania major* infection and interrogates the potential mechanistic contribution of activin signaling to host immune responses. Susceptible BALB/c and resistant C57BL/6 mice were subcutaneously infected with *L. major* promastigotes. At 8 weeks post-infection, parasite burden, macrophage polarization, and cytokine and immunoglobulin profiles, reflective of type I and type II immune responses, were assessed in draining lymph node (dLN), footpad and blood samples. Activin subunit (*InhBA*, *InhBB)* expression was quantified by RT-qPCR and activin A protein levels measured by flow cytometry in dLN and footpad tissues. *InhBA* mRNA and activin A protein levels were significantly elevated in dLNs of resistant C57BL/6 mice relative to susceptible BALB/c mice. These differences were less pronounced in the footpad tissue, suggesting tissue-specific regulation during infection. Activin A expression is associated with the resistant immune phenotype after *L. major* infection, implicating it as a candidate immunomodulatory marker of host resistance in CL. Functional studies employing receptor inhibition, neutralizing antibodies and/or gene silencing will be used to determine whether activin A mechanistically contributes to protective immunity, with implications for host-directed therapy strategies against CL.

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APPLICATION OF XENODIAGNOSIS IN VECTOR-HOST INTERACTION STUDIES: A PILOT FIELD EVALUATION IN SRI LANKA

Vidyani Kulatunga[1], Sanath Senanayake[1], Rajika Dewasurendra[1], Nilakshi Samaranayake[1], Manoja Kanakkahewage[2], Nadira D. Karunaweera[1]

[1]Department of Parasitology, Faculty of Medicine, University of Colombo, Colombo 08, Sri Lanka, [2]Department of Animal Production and Health, Southern Province, Sri Lanka, Matara, Sri Lanka

Xenodiagnosis is traditionally confined to laboratory settings. The present study is the first attempt to evaluate the feasibility of conducting xenodiagnosis on animals in their natural environment in Sri Lanka. Understanding the spread of *Leishmania* infection through vector-host interactions is important for the surveillance and control of Cutaneous Leishmaniasis (CL) in the country. This study was conducted in the high-prevalence districts of Matara and Hambantota. Laboratory-reared F1 progeny of *Phlebotomus argentipes* sandflies were used in this method. A total of 20 dogs and 14 cattle were randomly selected from these endemic areas. For each animal, 50 sandflies (at a female-to-male ratio of 1:2) were introduced using a mouth aspirator into a dark, conical xenodiagnosis cup temporarily secured to the animal's skin, where they remained for 30 minutes. After feeding, the sandflies were kept at 26°C and 80-90% humidity, with samples collected on days 4 and 8 and tested for Leishmania DNA via PCR. On average, 11.2 female sandflies per dog and 10.6 per cattle successfully fed, reflecting a feeding success rate exceeding 50%. This high rate suggests that the sandflies fed adequately, supporting the reliability of the xenodiagnosis method. While dogs had a slightly higher feeding success than cattle, the difference in the average number of blood-fed females was not statistically significant ($p > 0.05$). PCR testing revealed no *Leishmania* infection in any of the sandflies examined. Overall, this research successfully demonstrates that xenodiagnosis is feasible under natural field conditions, achieving consistent sandfly feeding on animal hosts and effectively applying real-world transmission dynamics. These results confirm the utility of the method as a valuable tool for epidemiological monitoring and for deepening the understanding of CL transmission in Sri Lanka's endemic regions.

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REASSESSING ARRHYTHMIA SCREENING IN CHAGAS CARDIOMYOPATHY: THE CLINICAL VALUE OF 24-HOUR HOLTER MONITORING

Marcelo Andres Buhezo Chamon[1], Shilah Faith Waters[2], Evan James Czulada[3], Adriana Hernani[1], Andrea La Ruta[4], Nicole La Ruta[1], Brianna Ortiz[5], Albornoz Geraldine[6], Hugo Armando Buhezo[7], Yazan Alshawkani[8], Robert H. Gilman[9]

[1]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [2]Johns Hopkins University, Millcreek, UT, United States, [3]Emory University School of Medicine, Atlanta, GA, United States, [4]PRISMA NGO, Baltimore, Plurinational State of Bolivia, [5]Universidad Catolica Boliviana, Santa Cruz, Plurinational State of Bolivia, [6]UNIFRANZ, Santa Cruz de la Sierra, Plurinational State of Bolivia, [7]PRISMA, Santa Cruz de la Sierra, Plurinational State of Bolivia, [8]Johns Hopkins Hospital, Baltimore, MD, United States, [9]Johns Hopkins Univ Bloomberg School of Public Health, Baltimore, MD, United States

Sudden cardiac death (SCD) is the leading cause of mortality in Chagas disease (CD), with current guidelines relying on standard 12-lead electrocardiogram (EKG) for risk stratification. While often unavailable in resource-limited settings, Holter monitoring (HM) is typically reserved for patients with unexplained syncope or at high risk for ventricular tachycardia (VT). This study's objective was to identify the arrhythmic burden associated with CD and compare the diagnostic accuracy of resting EKG versus HM. We performed a cross-sectional analysis within a large, prospective cohort between 2021 and 2024. 937 adults were included, consisting of 725 patients with seropositive CD and 212 seronegative controls. Clinical staging (A, B1, B2, C/D) was established using resting EKG and echocardiography. Multivariable logistic regression was utilized to evaluate predictors of arrhythmic risk. HM detected VT in 29 patients (3.1%), sinus pauses in 14 (1.5%), high-burden PVCs (>1000 PVCs/24 hours) in 44 (6.1%), while the diagnostic sensitivity of the resting EKG was only 0.0%, 0.0%, and 9.1% for each arrhythmia, respectively. Holter monitoring also detected premature ventricular contractions (PVCs) in 45.7% of patients compared to only 1.0% of patients who were detected by the resting EKG ($p < .001$). Multivariable analysis revealed that reduced left ventricular ejection fraction (LVEF < 50%) (aOR 15.2, 95% CI 6.8-33.7) and the presence of any PVC on resting EKG (aOR 8.9, 95% CI 1.3-60.0) were major independent predictors for discovering VT or a high PVC burden on HM. Stratification by disease stage showed that in Stage B1 the resting EKG identified PVCs in 1.0% of patients, whereas HM identified PVCs in 47.3% of the same group ($p < .001$). EKG underestimates the arrhythmogenic burden of arrhythmias associated with CD and may fail to detect the lethal ones in some asymptomatic patients. Current symptom-driven screening protocols therefore may inadequately identify patients at risk for disease progression, morbidity, and mortality. Routine HM use in patients with CD may help improve risk stratification and severe cardiac outcomes, such as SCD.

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MICRIA™: 2-MINUTE MAGNETIC CAPTURE RAPID IMMUNOASSAY PLATFORM FOR POINT-OF-CARE DETECTION OF INFECTIOUS DISEASES

Santosh George[1], Yevgeniy Studentsov[1], Alicia C. Majeau[1], Nadezhda Karaseva[1], Jeremy Schonhorn[1], Robert H. Gilman[2], Andrew E. Levin[1]

[1]Kephera Diagnostics, Framingham, MA, United States, [2]Johns Hopkins Univ Bloomberg School of Public Health, Baltimore, MD, United States

Point-of-care diagnostics are critical for the control of infectious diseases, particularly in resource-limited settings, yet current lateral flow assays are limited by development and manufacturing complexity, throughput, and variable performance. We have developed a novel platform, Magnetic Capture Rapid Immunoassay (MiCRIA™), which enables rapid and sensitive antibody detection using an innovative assay principle. Unlike conventional lateral flow assays, MiCRIA™ enables antigen-antibody binding on particles in solution prior to separation, followed by magnetic capture of immune complexes flowing through a capillary channel, eliminating the need for immobilized capture lines on the device itself. The capillary device is an inexpensive generic consumable with no biological components, and assay-specific reagents can be made in bulk as soluble particles. Readout is fluorescent via a low-cost compact reader. The capillary assay principal results in fast reaction kinetics, with a total assay time of 2-3 minutes. In a preliminary study, a MiCRIA™ assay was developed using 2 *T cruzi* peptide antigens to detect antibodies in Chagas disease patient sera confirmed by standard serology (concordant results on two independent assays). The MiCRIA™ assay detected 22/22 sera from Chagas patients from geographically diverse origins including Bolivia, Mexico and Honduras, with 0/20 false positives among blood donor controls. The MiCRIA™ assay also showed equivalent performance to an ELISA, and better specificity than a lateral flow assay, all using the same antigens. In separate studies, MiCRIA™ enabled detection of antibodies in finger-prick whole blood samples without prior plasma separation. Advantages of MiCRIA™ as a POC system are (1) assay development and scaled up production require only standard lab equipment, not specialized instrumentation as for lateral flow; (2) use of an inexpensive generic capillary cassette; and (3) 2 minute assay turnaround time. These features position MiCRIA™ as a broadly adaptable platform for rapid, scalable point-of-care diagnostics for neglected tropical diseases and emerging infections.

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ISOTHERMAL AND CRISPR-BASED MOLECULAR ASSAYS FOR *BABESIA* PARASITES

Nirmallya Acharyya, Sabari Nath Neerukonda, Anne Eder, Sanjai Kumar, Brendan Elsworth

FDA/CBER/OBRR, Silver Spring, MD, United States

Human babesiosis, caused by infection of the red blood cells with *Babesia* species parasites, is transmitted by ticks and, also by blood transfusion. Diagnosis is currently based on the presence of parasites in a blood smear by microscopy or by polymerase chain reaction (PCR) test which require trained technicians and specialized equipment. We have developed a *Babesia microti* specific, and a pan-babesia detection assay using isothermal recombinase polymerase amplification (RPA) combined with Cas12-based CRISPR (RPA-Cas12) and termed it BabRPACas-12. This assay detects parasite RNA and utilizes the pan-*Babesia* and *B. microti*-specific 18s rRNA sequences and possesses the analytical sensitivity of 4.0 copy of *B. microti* 18s rRNA gene. In whole human blood spiked with infected red blood cells, we have demonstrated sensitivity of 125 parasites/ml and 62.5 parasites/ml for *B. microti* and *B. divergens* parasites respectively. Clinical sensitivity and specificity of this assay is being determined in human babesiosis patients who are *B. microti* positive by microscopy/PCR and *B. microti* PCR positive asymptomatic blood donors from endemic areas in the United States. In addition to application in clinical diagnosis, this level of sensitivity would allow detection of window period cases post tick-exposure, and in identifying low-grade asymptomatic infections in surveillance and epidemiological studies. In summary, the BabRPA-Cas12 offers the potential as simple point-of-care diagnostic and in high-throughput surveillance studies, with the sensitivity and specificity of a PCR test and the ability to multiplex for the detection of other tickborne pathogens.

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PROSPECTIVE ANALYSIS OF CHAGAS DISEASE SEROLOGY TESTING FOR CLINICAL POPULATIONS PRESENTING TO A SAN FRANCISCO-BASED HOSPITAL SYSTEM

Abraham Soto[1], Rachel Alfaro Leone[1], Emily A. Kelly[1], Beatrice Marcelo[1], Colette J. Match[1], Barbara L. Haller[1], Caryn Bern[1], Carina Marquez[2], Jeffrey D. Whitman[1]

[1]University of California, San Francisco, San Francisco, CA, United States, [2]HIV/AIDS Division San Francisco General Hospital, San Francisco, CA, United States

Diagnostic challenges for chronic Chagas disease (CD) include a requirement for confirmatory testing by two assays and variable test performance characteristics. In this study, we prospectively analyze clinical CD testing in our hospital system to examine the performance of three serology tests: Chagas' Kit (Hemagen), Chagatest Recombinante v.3 (Wiener), and Chagas Detect Plus (InBios). With this information, we hope to better understand real-world test performance, as well as patient demographics and physician practices in our institution. Since Oct. 2024, our hospital has performed Hemagen and Wiener ELISAs in tandem for clinical CD testing. Remnant serum was used to perform the InBios lateral flow assay. Results were associated with patient data and ordering provider specialty. Contingency table analysis generated positive and negative percent agreement (NPA) for each assay compared to the consensus (2 out of 3) result. UCSF Institutional Review Board has approved this project. Testing has been analyzed for 79 patients. Two patients (2.5%) were reactive by all assays. NPA to the consensus result was 100% for Wiener, 97.0% for Hemagen, and 87.0% for InBios. The distribution of patients who had lived in an endemic country (top 5) was: Mexico (14%), El Salvador (13%), Nicaragua (11%), Guatemala (9%), Honduras (6%). Both positive patients were from El Salvador. Orders by clinical specialty include infectious diseases (35%), cardiology (32%), internal medicine (19%), family medicine (8%), other (5%), and rheumatology (1%). The number of positive patients matches our expected positivity rate from epidemiologic risk factors. NPA for these tests is consistent with previous studies, but requires more samples to increase statistical confidence. Most patients had ties to CD endemic regions and testing was primarily initiated by infectious diseases and cardiology, highlighting the importance of epidemiologic risk assessment with specialty-driven expertise. Our volume of testing is lower than expected, suggesting increased provider education for CD risk factors is essential for improving CD care in non-endemic settings.

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HOST TRANSCRIPTIONAL LANDSCAPE IN MURINE CUTANEOUS LEISHMANIASIS COMPLICATED BY MRSA

Qitong Wu[1], Marissa Macchietto[2], Zachary Blankenheim[3], Fernanda Fortes de Araujo[4], Hui Liu[1], Lynne Bemis[3], Naomi E. Aronson[5]

[1]Uniformed Services University of the Health Sciences, Bethesda, MD, United States, [2]University of Minnesota Supercomputing Institute, Minneapolis, MN, United States, [3]University of Minnesota, Medical School Duluth Campus, Duluth, MN, United States, [4]USUHS, Bethesda, United States, [5]USUHS, Bethesda, MD, United States

Cutaneous leishmaniasis (CL) causes chronic, ulcerating lesions and affects 1 million people globally. Ulcer dysbiosis—often with methicillin-resistant *Staphylococcus aureus* (MRSA)—is associated with delayed healing. *S. aureus* in a Th1 murine ear CL model promoted exacerbation by IL-1β–dependent inflammation. We hypothesized that the host immune response in a Th2 mouse base-of-tail (BOT) model of CL-MRSA differs. Ten BALB/c mice were infected subcutaneously with 10^7 metacyclic *Leishmania major* (Lm) at BOT, alongside 5 uninfected controls. MRSA was topically applied to 5 ulcers twice during week 4-5 post Lm. Lesion biopsies were obtained at week 8 for RNA sequencing. RNA was extracted using the Qiagen RNeasy kit. Libraries were prepared with the Illumina RiboZeroPlus Ligation workflow using the TruSeq stranded mRNA kit and sequenced on the Element Aviti platform. Reads were mapped to a combined reference including *Mus musculus*, *Staphylococcus aureus* USA300, Lm genomes for host-pathogen expression profiling. Differentially expressed genes (DEGs) were analyzed via gene set enrichment analysis. CL-MRSA lesions were 1.5-fold larger than CL alone. Compared with controls, Lm infection yielded 5710 DEGs (3630 up, 2080 down), while the CL-MRSA group showed 11428 (4810 up, 6618 down). Both groups showed robust activation of interferons and TNF- α signaling, indicating a shared immune signature. Lm infection uniquely activated antigen processing and presentation pathways, whereas CL-MRSA group suppressed pathways related to amino acid metabolism and MYC-associated transcriptional activities, with upregulation of oxidative-stress and pro-inflammatory responses. These transcriptional changes coincide with larger lesion areas, suggesting that MRSA in CL lesions both amplifies inflammatory pathways and dampens MYC-mediated responses. Lm infection alone showed activation of the antigen processing and presentation pathway, potentially due to impaired antigen presentation in the presence of MRSA.

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MICROTUBULE ASSOCIATED PROTEINS (MAPS): A NEWLY PROPOSED MULTIGENE FAMILY IN *TRYPANOSOMA CRUZI*

Martin Alan Winkler[1], Alfred A. Pan[2]

[1]Biotech Advisor, Lawrence, KS, United States, [2]TNTC, inc., Pleasant Hill, CA, United States

The *Trypanosoma cruzi* genome is highly complex, consisting of 50% repetitive sequences and large, multigene families. At present, there are six large gene families and each contains over 200 entities with a noteworthy number of pseudogenes from gene duplication and recombination. The most significant of these are involved in pathogenicity and avoidance of the host immune response. We have cloned GenBank® M21331 (Ag 36) from *T. cruzi* amastigotes. Its DNA sequence has a remarkable gene sequence identity to human immune genes (IFN-α, IFN-β, and IFN-γ), which indicates molecular mimicry of immune genes in humans by this protozoan parasite. The M21331 DNA sequence contains an internal sequence duplication that can result in an additional copy of the gene, and sometimes an increase in gene size after meiosis. We queried 30 available *T. cruzi* strains, whose

genomes had been completely sequenced, with the BLAST-p or the t(BLAST)n algorithms, using the translated M21331 sequence as a probe. Twenty strains (18 from humans and one each from Bat and Opossum) showed a total of 69 M21331 related genes. At least 32 of those M21331-related genes each contained multiple copies of the Ag 36 amino acid motif. Results of the six human Discrete Typing Units (DTUs) examined, indicate majority presence of M21331 in DTUs TcI//TcVI. However, M21331 was present in 50% (5/10) of DTU TcII human strains, possibly due that DTU's extensive genomic variability and complex aneuploidy patterns, and DTU TcIV strains did not exist in the databases. Of the 69 genes we detected, seven gene sequences with 100% amino acid sequence identity have been described as coding for "Microtubule Associated Proteins (MAPs)." The genes we now report are a large family of related genes, and therefore, a new multigene family in *T. cruzi*.

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DIAGNOSING AND TREATING CHAGAS DISEASE IN A NON-ENDEMIC SETTING: FINDINGS FROM A SMALL COHORT

Helen Mahoney West[1], Katherine McAleese[1], Endale Tefera Dejene[2], Molly Cuvellier[2], Carly Milliren[1], Leslie Scherl[3], Elizabeth Barnett[2], Julia Koehler[1]

[1]Boston Childrens Hospital, Boston, MA, United States, [2]Boston Medical Center, Boston, MA, United States, [3]Neighborhood Health Center, Boston, MA, United States

Chagas disease (CD) is a neglected disease of poverty endemic in the continental Americas withapproximately 300,000 cases in the US. Early screening and treatment of children prevents irreversible cardiac andgastrointestinal complications and onward congenital transmission. Therapy requires twice daily medication for 60days and is frequently complicated by adverse effects leading to treatment interruptions. We describe outcomes of anovel screening and treatment collaboration via the Strong Hearts project in Boston, Massachusetts for pediatric Chagas disease patients. We conducted a retrospective chart review of children with confirmed CD identified through primary care–based screening between March 2023 and March 2025. Demographic, clinical,laboratory, and management data were collected. Seventeen children aged 3–18 years (median 9.5 years; 59% male) among 6124 children screened, tested positive for Chagas disease. Birth locations included the United States, Central America, South America, the Caribbean; one U.S.-born child reported travel to an endemiccountry. All patients were prescribed benznidazole. Sixteen children completed the full course,with an average number of 9 encounters during treatment with infectious diseases clinicians, socialworkers, and pharmacists. Adverse events occurred in 75%, most commonly rash, pruritus, andnausea. Treatment interruptions occurred in 44% due to adverse effects, miscommunication, lossof insurance or missed doses. All patients who initiated benznidazole were able to complete treatment. Adverse events were frequent and, together with miscommunications, contributed to causing treatment interruptions. With significant clinical and social support, pediatric Chagas disease can be diagnosed in primary care and treated to prevent fatal outcomes.

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CYTOKINE-DRIVEN GLIAL ACTIVATION IN A MURINE MODEL OF *TRYPANOSOMA CRUZI* INFECTION

Mariel Burquez[1], Kathryn Jones[2]

[1]National School of Tropical Medicine Baylor College of Medicine, Houston, TX, United States, [2]Baylor College of Medicine, Houston, TX, United States

Trypanosoma cruzi (TC), the causative agent of Chagas disease (CD), is a vector-borne intracellular parasite affecting approximately 10 million people worldwide. While chronic infection is classically associated with cardiomyopathy and gastrointestinal dysfunction, increasing evidence suggests that central nervous system (CNS) involvement may occur even in immunocompetent individuals. However, the mechanisms underlying CNS pathology in CD remain poorly understood. CNS injury may result from direct parasite invasion or from systemic inflammation leading to blood-brain barrier (BBB) disruption. Glial cells, including astrocytes and microglia, are key regulators of neuroinflammation and may play a central role in CD-associated neuropathology. In this study, we examined neuroinflammatory responses in a murine model of TC infection, focusing on cytokine expression and glial activation. Quantitative PCR analysis demonstrated increased expression of pro-inflammatory mediators, including interleukin-6 (IL-6)and tumor necrosis factor-α (TNF-α), in infected animals. To further characterize CNS immune responses, immunohistochemical (IHC) analyses were performed. Morphological assessment of Iba1$^+$ microglia revealed changes consistent with activation, including increased soma size and altered cellular architecture. Notably, these changes occurred without significant increases in overall microglial density, suggesting functional activation rather than proliferation. These findings support a model in which TC infection induces a cytokine-driven neuroinflammatory environment that promotes glial activation and may contribute to BBB dysfunction and neuronal injury. Ongoing studies aim to further define astrocytic responses and neuronal damage markers, including GFAP and neurofilament light chain (NEFL).

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WHOLE-GENOME SEQUENCING OF FUNGAL PATHOGENS ISOLATED FROM FILARIAL LYMPHEDEMA WOUNDS IN SOUTHERN GHANA

Dzidzor Yawa Ayeke

West African Centre for Cell Biology of Infectious Pathogens, University of Ghana., Accra, Ghana

Lymphatic filariasis is frequently complicated by persistent wounds and recurrent infections that significantly impair quality of life in endemic communities. While bacterial infections in lymphedema-associated wounds have been widely studied, the fungal component of the wound microbiome remains poorly characterized. Fungal colonization may contribute to chronic inflammation and wound persistence, yet little is known about its genomic characteristics. This study aimed to characterize fungal pathogens associated with filarial lymphedema wounds using whole genome sequencing. A cross-sectional study was conducted among 100 individuals with filarial lymphedema from eight endemic communities in the Ahanta West District: Dixcove, Achowa, Busua, Butre, Asemkow, Ampatano, Princess Town and Akatakyi. Skin and wound swab samples were collected between December 2021 and February 2022 and cultured using standard mycological techniques. Fungal isolates were identified and genomic DNA was extracted. Whole genome sequencing was performed using Illumina platform. Genome assembly and annotation were used to assess fungal diversity, virulence determinants and antifungal resistance markers. Genomic analysis identified 18 fungal species belonging to nine genera, demonstrating considerable fungal diversity in lymphedema-associated wounds. Cladosporium (32.0%), Aspergillus (27.6%) were the most abundant genera, Periconia (13.3%), Penicillium (12.9%), and Candida (8.6%). Lasiodiplodia, Acrophialophora, Zygosporium, and Trichoderma were detected at lower frequencies (1.4% each). Whole genome sequencing revealed genomic variability among isolates and genes associated with fungal virulence and environmental adaptation. Markers linked to antifungal resistance were detected in selected isolates. Mixed fungal colonization was common, indicating a complex polymicrobial fungal community. These findings highlights the potential clinical significance of fungal colonization in chronic wound pathology and support integrating fungal genomics into wound surveillance and management strategies in endemic settings.

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INTEGRATION OF NEGLECTED TROPICAL DISEASE (NTD) INTO PRIMARY HEALTH CARE: OPERATIONALIZING NTD INTEGRATION WITHIN ETHIOPIA'S HEALTH SYSTEM

Getachew Gebreselassie Nida

Amref Health Africa, Black or African American, Ethiopia

Introduction: This abstract shares lessons from Ethiopia's initiative to integrate Neglected Tropical Disease (NTD) services into routine primary health care. Building on a multi-stakeholder planning workshop and roadmap across three pilot woredas, the initiative identifies barriers and solutions across health system building blocks. It highlights how integration, collaboration, plan alignment, and implementation research can strengthen sustainable NTD programming and health systems. The aim is to present Ethiopia's approach to integrating NTD services into primary health care and share lessons from a national process moving beyond vertical campaigns toward system-based delivery. NTDs disproportionately affect marginalized communities and are linked with poverty, poor sanitation, limited access to services, and weak systems. Despite progress, the response remains reliant on periodic mass drug administration and externally funded programs, posing sustainability challenges. A multi-stakeholder workshop involving the Ministry of Health, regional bureaus, and partners identified pathways for integrating NTD services into routine PHC across three woredas. Using the WHO health system framework, participants analyzed barriers across service delivery, workforce, supply chain, financing, governance, community engagement, and information systems, while mapping existing interventions. Findings showed services remain campaign-driven with limited routine care. Health workers lack training, supply systems are not fully integrated, financing is limited, and indicators are weakly reflected in DHIS2. Enablers include the health extension program, MDA platforms, PHC structures, and digital systems such as eCHIS. Priorities include integrated service packages, workforce strengthening, supply chain integration, improved financing, multisectoral collaboration, and community engagement. In conclusion, the initiative provides insights for shifting from vertical programs to integrated systems, supporting universal health coverage and sustainable NTD programming.

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DONOR-ENABLED SUSTAINABILITY PLANNING FOR NEGLECTED TROPICAL DISEASE SERVICES (2018-PRESENT): A POLICY ANALYSIS OF USAID'S EVOLVING APPROACH

Portia Aning[1], Margaret C. Baker[2], Aarman Khayat[1], Justin Tine[3], Andrew Paul Kyambadde[4], Emily Wainwright[5]

[1]Georgetown University, Washington, DC, United States, [2]Georgetown University, VASHON, WA, United States, [3]Independent Consultant, Milan, Italy, [4]Ashburn, VA, United States, [5]U.S. Agency for International Development, Hyattsville, MD, United States

Since the early 2000s, substantial progress has been achieved in addressing Neglected Tropical Diseases (NTDs), particularly those targeted with preventative chemotherapy. In 2026, the World Health Organization reported that 58 countries had eliminated at least one NTD. These successes relied on vertically structured programs with high levels of external donor support. To build on these efforts, WHO's 2030 NTD Roadmap emphasizes the need to strengthen country ownership with more integrated services for all NTDs. From 2006 until its closure in 2025, USAID supported NTD programs in over 30 countries, supporting 3.3 billion preventative treatments. While this support was primarily focused on mapping, mass drug administration, and surveillance to achieve elimination targets, a new sustainability strategy was introduced in 2018. This approach supported country-led efforts to develop and implement Sustainability Plans aimed at embedding NTD prevention and treatment services within national systems - across health and other sectors. A multi-country research study analyzed the evolution of this approach. A retrospective qualitative case study design was used, guided by the Walt and Gilson policy analysis framework that includes policy content, process, actors, and context. Data sources included in-depth interviews with key informants and over 100 project documents spanning from 2018 to 2025 (including USAID strategy documents, partner country workplan and reports, national sustainability plans, and policy documents). Both documents and interview transcripts were coded thematically in MAXQDA, a qualitative research software package. Findings highlight key learning and adaptations made over the seven-year period, compare cross-country results, and identify key drivers of success. This study offers a roadmap for future NTD and other disease-focused initiatives seeking to enhance long-term country ownership and optimize existing systems.

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THE LEISHMANIASES IN SUDAN AND SOUTH SUDAN: A SCOPING REVIEW

Adriana Totten[1], Katherine O'Brien[1], Sofia Wojtaszek[1], Riley Fanning-Hughes[1], Philip Espinola Coombs[2], Dia-Eldin Elnaiem[3], Lexon Mabrouk Manibe[4], Ermias Diro Ejara[5], Richard G. Wamai[6]

[1]Northeastern University, Boston, MA, United States, [2]Simmons University, Boston, MA, United States, [3]Univ of Maryland Eastern Shore, Princess Anne, MD, United States, [4]Atlantic International University, Honolulu, HI, United States, [5]University of Antwerp, Antwerp, Belgium, [6]Northeastern Univ, Boston, MA, United States

The leishmaniases are a group of neglected tropical diseases endemic in 99 countries, with visceral leishmaniasis (VL) and cutaneous leishmaniasis (CL) representing the two main clinical forms. Geographically spread in parts of southeast Asia and south America, eastern Africa bears the largest share of the leishmaniases particularly in Sudan and South Sudan. In 2024, the World Health Organization released a strategic framework targeting VL elimination in East Africa by 2030, emphasizing the urgency of identifying national research priorities and gaps. However, country-specific evidence to guide elimination efforts remains sparse. Ongoing conflict in both countries has disrupted disease surveillance, control, and treatment infrastructure, undermining progress. This study aims to map the existing evidence base for leishmaniasis in Sudan and South Sudan, identify research gaps, and support evidence-informed elimination planning in both countries. These two scoping reviews are underway following a protocol developed in accordance with the PRISMA-ScR guidelines, adapted from this team's scoping reviews previously applied to Kenya and Ethiopia, and published in 2024. Multiple electronic databases were searched for relevant articles. Study selection proceeded through title and abstract screening, full-text review, and data extraction, each conducted by two independent reviewers, with a third reviewer resolving conflicts. Approximately 700 studies were selected for inclusion in the review across both countries. The data extraction phase is on-going, where studies are assigned specific study themes and categories using a standardized codebook previously published by the research team. These reviews will produce a structured synthesis, identify leishmaniasis research gaps, and generate recommendations for Sudan and South Sudan, providing a foundation for prioritizing future research and informing national and regional strategies aligned with the 2030 elimination targets. This study is expected to be complete by July 2026, and findings will be widely disseminated in Sudan, South Sudan, and among relevant stakeholders.

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COST AND COST-EFFECTIVENESS OF INTEGRATING MASS DRUG ADMINISTRATION FOR HELMINTH CONTROL WITH SEASONAL MALARIA CHEMOPREVENTION IN GHANAIAN SCHOOL-AGED CHILDREN

Theresa Tawiah[1], Lucy Paintain[2], Dennis Adu-Gyasi[1], Mathilda Tivura[1], Samuel Adusi[1], Antony I. Siibu[1], Elvis Wilson[1], Richmond Kessie[1], Elisha Adeniji[1], Eliezer Ofori Odei-Lartey[1], Stephaney Gyaase[1], Richard Tetteh[1], Paul Snell[2], Brian Greenwood[2], Daniel Chandramohan[2], Kwaku Poku Asante[1], Muhammed Olanrewaju Afolabi[2]

[1]Kintampo Health Research Centre, Kintampo, Ghana, [2]London School of Hygiene & Tropical Medicine, London, United Kingdom

Overlapping geographical distribution of malaria and helminth infections in Ghana presents an opportunity for integrated control strategies. Currently, anthelmintic mass drug administration (MDA) is delivered annually to school-aged children and seasonal malaria chemoprevention (SMC) is given monthly to children under-five years for four peak transmission months. Results from recent studies suggest a need to extend SMC delivery to children over-five years. Integrating expanded SMC delivery with MDA could be an efficient strategy to improve health outcomes. A model-based economic evaluation was conducted from the provider perspective to estimate cost and cost effectiveness of integrating anthelmintic MDA with expanded SMC delivery among children <10years in Pru East district of Ghana. Ingredients-based costing estimated financial resources required to deliver SMC and MDA separately and together. Costs included personnel, drugs and supplies, training, transport, supervision and community sensitization. A decision analytic model compared integrated expanded SMC+MDA with current practice over one transmission season. Health outcomes were expressed as averted malaria cases, anaemia cases and DALYs. All costs reported in 2024 US dollars. Annual financial costs for standalone delivery were estimated at $33,863 for MDA, $81,801 for SMC in children under-five and $82,553 for SMC in children 5-10years, a total of $198,216. Integrated delivery reduced costs to $182,605, saving $15,611. Average annual cost per child targeted was $9.16 when interventions were delivered separately, compared with $3.38 under integrated delivery. More detailed information on additional DALYs averted with the combined intervention and the incremental cost effectiveness ratio per DALY averted will be presented at the meeting. Integrated delivery of MDA with expanded SMC in Pru East is a cost saving strategy compared with separate implementation. Overall costs still increase because SMC is expanded to older children, but some savings can be gained and the average annual cost per child is substantially reduced.

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SCALING SUSTAINABLE DENGUE SOLUTIONS: INSIGHTS FROM VIETNAM'S EXPERIENCE

Hoai Thu Nguyen Mong, Huong Nguyen, Minh Ngoc Nguyen

Pasteur Institute in Ho Chi Minh City, Ho Chi Minh, Vietnam

The World Health Organization has identified dengue as a priority disease in achieving the Sustainable Development Goals by 2030. In Vietnam, 190,040 confirmed cases were reported in 2025, representing a 28.4% increase compared to 2024. Risk factors are linked to social determinants, environmental conditions, and climatic variability. We document interventions, challenges, and recommendations for strengthening dengue prevention and control in Vietnam. We reviewed national dengue and vector control strategies, alongside expert assessments of implementation. Monthly vector index monitoring was conducted to generate provincial Breteau index trends. Integrated surveillance of Dengue, Chikungunya, and Zika confirmed the presence of A. aegypti and A. albopictus, with common breeding sites including jars, tanks, and ponds. Community participation remained a significant challenge. Pyrethroid-based interventions, including Olyset nets and indoor spraying, reduced mosquito populations but were constrained by emerging insecticide resistance. Vietnam is among 12 countries piloting the release of Wolbachia-infected mosquitoes, which produced rapid declines in dengue incidence. Effective mosquito control requires an integrated strategy combining chemical, physical, and biological methods to achieve sustainable outcomes. Future interventions should prioritize strengthening community engagement through ongoing training, enhancing monitoring and evaluation with technology-based tools, and expanding innovative approaches such as Wolbachia programs and spatial repellents. Public awareness campaigns and community-based education are essential. TAK-003, approved in 2024, and V181, currently in clinical trials, offer promising vaccine-based support for comprehensive control programs in the coming years. Dengue continues to pose a major public health burden in Vietnam, with rising incidence despite conventional vector control measures. Sustainable strategies - including health education, community-based interventions, and vaccines - are essential components of long-term dengue prevention and control.

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AN OBSERVATIONAL STUDY TO TEST THE POTENCY OF GLP GRADE PHENOL FREE LYOPHILIZED LEISHMANIN ANTIGEN FORMULATION

Sreenivas Gannavaram[1], Shin-Ichi Inoue[2], Swarnendu Kaviraj[3], Sanjay Singh[4], Lucas P. Carvalho[5], Edgar M. Carvalho[6], Camila De Oliveira[7], Abhay R. Satoskar[8], Shinjiro Hamano[9], Hira L. Nakhasi[10]

[1]US Food and Drug Administration, Silver Spring, MD, United States, [2]Nagasaki University, Nagsaki, Japan, [3]Gennova Biopharmaceuticals, Pune, India, [4]Gennova Biopharmaceuticals Ltd, Pune, MH, India, [5]Federal Univ of Bahia, SALVADOR, BA, Brazil, [6]Federal University of Bahia, Salvador, Brazil, [7]Centro de Pesquisas Gonçalo Moniz, FIOCRUZ, Salvador, Brazil, [8]Ohio State University, Department of Pathology, Columbus, OH, United States, [9]Inst Trop Med, Nagasaki Univ, Nagasaki, Japan, [10]FDA/CBER/OBRR, Silver Spring, MD, United States

Leishmaniasis is a vector-borne disease transmitted through infected sand fly vectors . The leishmanin skin test (LST), used to detect exposure to Leishmania parasites, is no longer available due to lack of well characterized antigens. Towards reintroducing the LST, we previously developed a preparation of the leishmanin antigens using a freeze-thaw (FT) process, which induced a delayed-type hypersensitivity (DTH) response in hosts exposed to Leishmania parasites. The storage of FT preparation antigens requires refrigeration, which is not practical for use in field settings. We developed a safe, simple and scalable antigen extraction process and a phenol-free lyophilized-LST antigen formulation that obviates the need for a cold-chain under Good Laboratory Practice conditions (GLP-LSTLyo). The immunogenicity of GLPLSTLyo was evaluated in murine models of Leishmania infections in support of two distinct use cases: (i) to detect latent infections of Leishmania in endemic areas and (ii) as a surrogate of vaccine-induced immunogenicity. Towards this goal, legitimatization with wild-type Leishmania major and immunization of mice with the live-attenuated L. major vaccine strain lacking the centrin gene (LmCen-/-) were used as animal models. The GLP-LSTLyo antigen was administered, and its ability to induce a DTH response was monitored for 48 hours. Additionally, the potency of the GLP-LSTLyo was assessed by immune phenotyping of the cells isolated from the DTH tissue via flow cytometry. Only the DTH sites from pre-exposed animals, but not the excipient controls, showed enrichment for predominantly CD8+ T cells, along with CD4+ T cells and macrophages, consistent with previous studies. Additionally, in an interferon gamma release assay (IGRA), stimulation of whole blood collected from healed, active cutaneous leishmaniasis (CL) cases in a Leishmania endemic area in Brazil with GLPLSTLyo induced IFN-γ and IL-10. These results demonstrated the potency of our formulation in IGRA studies, which could aid immunogenicity studies in vaccine trials, and surveillance studies in both endemic and emerging areas of Leishmania infection.

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A CHIMERIC ANTIBODY APPROACH TO IMPROVE STANDARDISATION OF SEROLOGICAL DIAGNOSTICS FOR SCRUB TYPHUS.

Puntanat Tattiyapong, Suphasuta Khongpraphan, Jantana Wongsantichon, Stuart D. Blacksell

Mahidol Oxford Tropical Medicine Research Unit, Bangkok, Thailand

Orientia tsutsugamushi, the causative agent of scrub typhus, remains a significant contributor to the burden of neglected tropical diseases across the Asia-Pacific. The disease typically presents as an undifferentiated febrile illness, often leading to diagnostic delays and increased mortality. Serological assays, including indirect immunofluorescence assay (IFA) and enzyme-linked immunosorbent assay (ELISA), remain the mainstay for diagnosis. While IFA is the reference method, it requires high-level biosafety infrastructure for antigen preparation, limiting its utility in resource-limited regions. ELISA offers a more practical and higher-throughput alternative. Recently, Luminex multiplex assays have emerged due to their ability to detect multiple targets from a single sample. However, their performance is hindered by reliance on patient-derived sera as positive controls, which are limited in availability, variable between batches, and prone to cross-reactivity and antigenic variability. To address these challenges, this study developed chimeric monoclonal antibodies (mAbs) targeting the conserved 56 kDa type-specific antigen (TSA56) of *O. tsutsugamushi* as standardised positive controls for Luminex assays. Mouse mAbs were generated using hybridoma technology, and their variable regions were cloned into vectors containing human IgG constant regions. The chimeric mAbs were expressed in HEK293T cells and confirmed by dot blot. Binding specificity and cross-reactivity were evaluated using a Luminex assay against a panel of febrile illness antigens and compared with positive human sera. The chimeric mAbs demonstrated reproducible production, strong specificity for target antigens, and no detectable cross-reactivity with non-target pathogens. This study highlights a scalable strategy for generating reliable and renewable positive controls that can replace patient-derived sera. Beyond scrub typhus, this approach is broadly applicable to other infectious diseases, offering a pathway to improve assay consistency, quality control, and inter-laboratory comparability in serological diagnostics.

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EVALUATING DRIED BLOOD SPOTS FOR SEROLOGICAL ANTIBODY DETECTION USING LUMINEX ASSAY: A SCRUB TYPHUS PROOF-OF-CONCEPT

Suphasuta Khongpraphan, Puntanat Tattiyapong, Asama Vinitsorn, Surakit Wongwanna, Carlo Perrone, Yoel Lubell, Jantana Wongsantichon, Stuart D. Blacksell

Mahidol Oxford Tropical Medicine Research Unit, Bangkok, Thailand

Scrub typhus, caused by *Orientia tsutsugamushi*, remains a significant public health concern, particularly in the Asia-Pacific region. Detection of antibody responses is essential for sero-epidemiological studies, but conventional assays rely on serum obtained by venipuncture, which requires trained personnel and specialized equipment, limiting large-scale implementation. Dried blood spots (DBS) offer a practical alternative due to simplified collection, storage, and transport, requiring only minimal blood volume. However, their comparability with serum-based measurements has not been fully established. This study evaluated the performance of DBS for IgG detection using a Luminex bead-based assay. Matched DBS and serum samples (n = 201) from the SEACTN project were analyzed against *O. tsutsugamushi* strains Gilliam, Kato, and Karp, with antibody responses measured as median fluorescence intensity (MFI). DBS measurements showed strong agreement with serum, with high correlations for Gilliam (r = 0.99), Kato (r = 0.99), and Karp (r = 0.98). Bland-Altman analysis showed minimal bias, with mean differences of 170.2, 149.8, and 182.9 MFI, respectively. Gilliam showed narrower limits of agreement (LOAs), ranging from -436.3 to 776.7, indicating consistent measurements, whereas Karp exhibited wider LOAs from -538.8 to 904.6, reflecting greater variability between sample types. However, Passing-Bablok regression indicated proportional differences between methods, with DBS tending to yield lower values at higher signal intensities. Despite these differences, DBS is a reliable and robust alternative to serum for Luminex-based serological assessment of scrub typhus. Its ease of collection and minimal processing requirements make DBS well-suited for large-scale surveillance, especially in resource-limited settings, with implications extending beyond scrub typhus to other infectious diseases.

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FROM MASS TREATMENT TO ADAPTIVE TARGETING: EVIDENCE FROM KENYA ON OPTIMIZING DEWORMING STRATEGIES IN DECLINING PREVALENCE SETTINGS

Chrispin Omondi Owaga[1], Mark Minnery[2]

[1]Evidence Action, NAIROBI, Kenya, [2]Evidence Action, Washington, WA, United States

As soil-transmitted helminth (STH) prevalence declines, national programs must transition from universal mass drug administration to more targeted strategies while maintaining program effectiveness. We evaluated adaptive treatment approaches within Kenya's national school-based deworming program using longitudinal program and survey data from 2012 to 2023. Following a decade of implementation that reduced national STH prevalence to approximately 5.8%, treatment strategies were progressively refined based on epidemiological evidence and spatial heterogeneity. The annual treatment population was reduced from approximately 6 million children at program inception to approximately 3 million by 2023, reflecting a shift from blanket coverage to targeted delivery. Analysis of program performance indicates that this transition did not compromise treatment coverage or delivery quality, with average coverage maintained at approximately 75% in targeted populations. Sub-national variation in prevalence remained substantial, necessitating differentiated approaches across counties and sub-counties. The adaptive model leveraged routine monitoring data, school-based delivery systems, and strengthened coordination between health and education sectors to maintain efficiency while responding to changing epidemiological conditions. These findings demonstrate that large-scale deworming programs can transition to targeted strategies without loss of effectiveness while improving resource efficiency. Kenya's experience provides a practical framework for countries seeking to optimize program design and sustain gains as they move toward elimination of STH as a public health problem

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TEMPORAL TRENDS AND REGIONAL DISTRIBUTION OF LEPROSY IN MEXICO: A NATIONAL ECOLOGICAL ANALYSIS, 2003-2023

Edgar Villarreal-Jimenez[1], Nina Mendez-Dominguez[2], Adrian Perez-Navarrete[2], Miriam Gamboa-Candila[1]

[1]Universidad Autonoma de Yucatan, Merida, Mexico, [2]Hospital Regional de Alta Especialidad de la Peninsula de Yucatan IMSS-Bienestar, Merida, Mexico

Leprosy is a disease caused by the bacillus *Mycobacterium leprae* and by *M. lepromatosis*. Mexico has been described as one of the countries with a high number of leprosy cases in Latin America; moreover, there are regions within the country with a high frequency of cases. Although a global decrease in cases has been reported, in Mexico it has not been clearly identified whether this downward trend is consistent across different sociodemographic characteristics and regions of the country. Our objective was to identify trends in leprosy cases in Mexico between 2003 and 2023. An ecological time series study was performed based on open-access data from the Annual Morbidity Database from 2003 to 2023 included variables were age group, sex, state of residence and region, and month of report. A descriptive analysis was performed using frequencies and percentages. States were grouped according to the five economic regions. Incidence was calculated using the projected population. Trends were analyzed using Join point regression to estimate Annual Percent Change (APC) with 95% confidence intervals (CI). Analyses are presented by age, sex, and region.

A total of 4,109 cases were reported in Mexico between 2003 and 2023, with a cumulative incidence rate of 3.11 per 100,000 inhabitants. Overall incidence decreased significantly (APC -5.39; 95% -5.81 to -4.95) during the period studied. Men exhibited a higher incidence (3.81 per 100,000) and also age ≥60 years (9.67 per 100,000). Leprosy affected the population residing in the northern region more (6.91 per 100,000). Declines were more pronounced among men (APC –6.01; 95% CI -6.78 to -5.20), individuals aged 45–59 (APC –7.56; 96% CI -7.56 to 6.69), though heterogeneity persisted between subgroups. In Mexico, trends in leprosy incidence decreased in the last two decades; however, this decline is not uniform across age groups or regions. It is important to strengthen prevention efforts for all Mexican region, particularly among the most vulnerable populations.

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STRENGTHENING NTD CONTROL THROUGH DISABILITY-INCLUSIVE WASH: BASELINE EVIDENCE FROM FOUR COMMUNITIES IN ABUJA, NIGERIA

Juliana Ajuma Amanyi-Enegela[1], Joseph Kumbur[2], Rinpan Ishaya[3], Moses Aderogba[4], Louise Makau-Barasa[5]

[1]CBM, Cambridge, United Kingdom, [2]Christoffel Blinden Mission Internaitonal, [3]HANDS, Jos, Nigeria, [4]The END Fund, Abuja, Nigeria, [5]The END Fund, New York, NY, United States

Schistosomiasis is a neglected tropical disease affecting over 200 million people worldwide. Nigeria carries a substantial burden, with an estimated 29 million infected across all 36 states, with school-aged children at the highest risk.In Nigeria's Federal Capital Territory (FCT), baseline surveys (2010-2014) reported prevalences of 7-52%, and preventive chemotherapy with praziquantel began in 2014. Suspected outbreaks in 2020 and persistently high prevalence underscore the need for integrated approaches beyond drug distribution. A mixed-methods study was conducted in four NTD-endemic communities across Abuja Municipal and Gwagwalada Area Councils. Structured questionnaires were administered to 188 systematically sampled households, supplemented by focus group discussions and key informant interviews exploring WASH behaviours and barriers for persons with disabilities. Key findings: 20% of households reported schistosomiasis, and 15% reported STH. While 60% had improved water access, distance and collection time remained barriers to consistent use. Only 35% had improved latrines, with regular use rarely observed. Hygiene practices were inconsistent: 65% washed hands after defecation, but only 55% did so after handling children's faeces. Water storage was inadequate, with only 45% keeping containers kept clean and covered. Higher income and literacy correlated with better WASH access, while larger households and marginalised groups faced greater barriers. Persistent WASH gaps are likely sustaining schistosomiasis and STH transmission in FCT communities. Priorities include expanding access to water and sanitation, tailoring health education to low-literacy populations, and adopting inclusive approaches for persons with disabilities and youth. Community co-design and routine monitoring of NTD and WASH indicators are recommended.

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IDENTIFYING 'WORMY' HOUSEHOLDS: A ONE HEALTH MIXED-METHODS STUDY ON SOIL TRANSMITTED HELMINTHS IN RURAL SOUTHERN INDIA

Gideon John Israel[1], Malathi Manuel[1], Ajithkumar Muthukumar[1], Manigandan Sekar[1], Vinothini Vijayavenkatesan[1], Zayina Zondervenni[1], Uniqueky Gratis Mawrie[2], Rohan Michael Ramesh[1], Nils Pilotte[3], Judd L. Walson[4], Amy J. Pickering[5], Kumudha Aruldas[1], Rajiv Sarkar[2], Sitara Swarna Rao Ajjampur[1]

[1]Christian Medical College, Vellore, TN, India, [2]Indian Institute of Public Health, Shillong, ML, India, [3]Quinnipiac University, Northampton, MA, United States, [4]The Johns Hopkins University, Baltimore, MD, United States, [5]University of California, Berkeley, CA, United States

Soil-transmitted helminths (STH) are a major public health problem in low-resource settings but the role of animal and environmental reservoirs in transmission remains unclear, particularly in rural communities. We conducted a One Health mixed-methods study in 597 household across 186 villages located in Timiri rural and Jawadhu hills tribal area, Tamil Nadu, India. Human stool (n=2096), animal stool (n=749), and household soil (n=597) were collected and tested by qPCR. STH prevalence was 26.0% (95% CI 24.3-27.7) in humans, primarily *Necator americanus* (25.9%). Goats had 7.5% (95% CI 3.6-14.7) of STH, primarily *Ancylostoma caninum* (3.7); pigs had 44.0% (95% CI 22.3-68.3) of STH, primarily *N. americanus* (28.0%), followed by *A. caninum* and *Ascaris lumbricoides* (12.0% each); dogs had 86.3% (95% CI 77.5-91.9) of STH, primarily *A. caninum* (81.3%), followed by *N. americanus* (25.0%). In addition, stray pigs and dogs sampled in these villages had 61.9% (95% CI 54.9-68.5) and 76.1% (95% CI 71.4-80.3) of STH. In household soil, STH was detected in 50.7% (95% CI 45.3-53.3), primarily *A. caninum* (43.4%), followed by *N. americanus* (13.4%). Overall, 45.5% households were "wormy" defined as positive for any STH in ≥2 sample types. Mixed-effects logistic regression identified household factors such as natural flooring (aOR 2.2, 95% CI 1.1-4.7), family size ≥5 (aOR 2.0, 95% CI 1.3-3.0), manure fertilizer use (aOR 1.7, 95% CI 1.1-2.6) and village characteristics such as moderate to high population density (aOR: 2.6/2.4, 95% CI 1.1-5.9/>1.0-5.8), high pig-to-human ratio (aOR 3.5, 95% CI 1.3-9.8), having stray pigs that were STH positive (aOR 3.9, 95% CI 1.3-11.7) as risks of being "wormy" household. Qualitative family interviews with 20 households revealed lack of awareness about bi-directional human-animal STH transmission; misconceptions that animals are 'clean', as a result not use personal protective equipment during routine animal care. High multi-host STH prevalence in rural, agrarian communities underscores the need to address animal and environmental reservoirs with integrated One Health intervention strategies for STH control and elimination.

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FROM FARM TO JOINT: A SCOPING REVIEW OF CAMPYLOBACTER-ASSOCIATED REACTIVE ARTHRITIS ACROSS THE ONE HEALTH SPECTRUM

Amanda K. Irish, KariAn E. Mulford, Cale B. Dobson, Jessica L. Dennis, Thiru Vanniasinkam, Karen I. Cyndari

University of Iowa, Iowa City, IA, United States

Campylobacter species are a leading bacterial cause of gastroenteritis worldwide and a recognized trigger of reactive arthritis (ReA), yet the epidemiology, clinical spectrum, and outcomes of Campylobacter-associated ReA remain poorly characterized. We conducted a scoping review to map the existing literature on Campylobacter-associated ReA and to identify critical knowledge gaps through a One Health conceptual framework linking animal reservoirs, foodborne transmission pathways, and post-infectious human disease. A systematic search of PubMed and Web of Science yielded 30,129 articles, which were screened through sequential stages of duplicate removal (n=11,178), filtering for English language and primary source (n=4,500), scope and data alignment review (n=473), and relevance assessment (n=78), resulting in 35 articles selected for final inclusion. Included studies spanned case reports, case series, cohort studies, and outbreak investigations across diverse geographic settings. Preliminary findings reveal substantial heterogeneity in case definitions, diagnostic criteria, and reported outcomes for Campylobacter-associated ReA, limiting cross-study comparison and accurate incidence estimation. The existing literature is heavily weighted toward high-income country settings despite the disproportionate global burden of campylobacteriosis in low- and middle-income countries where ReA likely represents an underrecognized sequela. Notably absent from the current evidence base is integration of upstream zoonotic transmission data — including Campylobacter strain characterization from animal reservoirs and food production systems — with downstream human clinical outcomes. This review highlights the need for standardized case definitions, prospective surveillance incorporating both microbiologic and rheumatologic outcomes, and a One Health approach that connects agricultural source attribution to post-infectious sequelae in order to reduce the global burden of Campylobacter-associated reactive arthritis.

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GENOMIC SURVEILLANCE OF MULTIDRUG-RESISTANT BACTERIA AND ANTIMICROBIAL RESISTANCE GENES IN URBAN-IMPACTED RIVERS: THE NEED FOR A ONE HEALTH AMR SURVEILLANCE APPROACH

Berhanu Yitayew Sahilu[1], Yimtubezinash Woldeamanuel[1], Daniel Asrat[1], Adane Mihret[2], Abraham Aseffa[2], Per-erik Olsson[3], Jana Jass[3]

[1]Addis Ababa University, Addis Ababa, Ethiopia, [2]Armauer Hansen Research Institute, Addis Ababa, Ethiopia, [3]Örebro University, Örebro, Sweden

Antibiotic-resistant bacteria in aquatic environments pose a serious One Health threat, with fecal waste and chemical pollutants driving their emergence and spread. This study investigated genomic surveillance of multidrug-resistant bacteria and antimicrobial resistance genes (ARGs) in urban-impacted Akaki River. Water samples from five sites along the river were analyzed for bacterial pathogens and ARGs. ARGs diversity and abundance were assessed using qPCR. Bacterial pathogens were isolated on selective agar, identified by MALDI-TOF mass spectrometry, tested for antibiotic resistance, and further characterized by whole-genome sequencing and PCR. Genes associated with resistance to fluoroquinolones, aminoglycosides, β-lactams, macrolides, tetracyclines, and vancomycin, as well as genes for multidrug and tetracycline efflux pumps, were detected. The majority of these ARGs were found near sites of anthropogenic activity, including hospitals and irrigation areas. Sites proximate to hospitals exhibited the highest abundance of ARGs. *Escherichia coli* strains (168) were most frequently resistant to erythromycin (163, 97%), doxycycline (132, 78.5%), tetracycline (127, 75.6%), and amoxicillin (127, 75.6%). Resistance to cephalosporin and carbapenem was 7.9% and 5%, respectively, with 80.9% of *E. coli* isolates being MDR. The β-lactamase genes were the commonly detected resistance genes in *E. coli*. The most prominent plasmid sequence replicons detected were from the IncF family (65%), Col (10%), and IncX3 (7.5%). The *Aeromonas* species (163) were resistant to a wide range of antibiotics with the highest being resistant to amoxicillin (144, 100%), ampicillin (142, 99%), amoxicillin/clavulanate (117, 81%), imipenem (75, 52%), ertapenem (132, 92%) and doxycycline (134, 93%). These findings indicate that the Akaki River serves as a reservoir and conduits for the dissemination of antibiotic-resistant bacteria and ARGs. The prevalence of resistance in clinically relevant bacterial pathogens underscores the critical need for integrated, One Health-based surveillance and containment strategies for AMR.

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ASSESSING THE GENETIC RELATEDNESS BETWEEN HUMAN AND ANIMAL DERIVED SOIL-TRANSMITTED HELMINTH ISOLATES FROM A ONE HEALTH STUDY IN INDIA

Malathi Manuel[1], Gideon John Selvapandian Israel[2], Uniqueky Gratis Mawrie[3], Nils Pilotte[4], Rajiv Sarkar[5], Sitara SR Ajjampur[6]

[1]Christian Medical College, Vellore, Tamil Nadu, India, [2]Christian Medical College, Vellore, India, [3]Indian Institute of Public Health, Shillong, India, [4]Quinnipiac University, Northampton, MA, United States, [5]Indian Institute of Public Health Shillong, ML, India, [6]Christian Medical College, Vellore, Vellore, India

Host-specificity among soil-transmitted helminths varies and has implications for zoonotic transmission and control strategies. Cross-species infection has been reported for *Ascaris* spp., whereas *Necator americanus* is primarily human-adapted, and *Ancylostoma ceylanicum* is a zoonotic hookworm. We conducted a one-health study collecting stool samples from humans, household and stray animals (dogs, goats and pigs) and soil samples across 597 and 444 households in rural and tribal areas of Tamil Nadu and Meghalaya, India, respectively. Using species-specific qPCR, we found *N. americanus* in humans (20.6%), dogs (16.7%), pigs (27.7%) and goats (6.3%); *A. lumbricoides* in humans (1.3%), pigs (23.5%) and goats (2.6%); and *A. ceylanicum* in humans (2.2%) and dogs (9.2%). However, the question of whether these animals are true hosts capable of sustaining infection or only transient carriers remains unknown. To investigate this further, we are examining the genetic relatedness between human and animal-derived isolates within the same villages using targeted amplicon sequencing of the mitochondrial large subunit ribosomal DNA (rrnL gene) to identify single-nucleotide polymorphisms (SNPs), define haplotypes, and construct haplotype networks. The detection of shared mitochondrial haplotypes between humans and animals within the same village would provide evidence for cross-species transmission, whereas consistent phylogenetic clustering by host species, with clear genetic differentiation between human and animal-derived isolates, would support host-restriction of human strains in non-human hosts. These findings will help distinguish between true reservoir hosts and incidental transport hosts, providing critical evidence to inform One Health control strategies.

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SEROPREVALENCE OF NAEGLERIA FOWLERI AMONGST UNIVERSITY OF SOUTH CAROLINA STUDENTS

Madeline Toth[1], Namya Gupta[1], Kennedy Flynn[2], Kia Zellers[1], Madeleine Meyer Torelli[1], Brandon D. Hollingsworth[3]

[1]University of South Carolina, Columbia, SC, United States, [2]Columbia, SC, United States, [3]Cornell University, Columbia, SC, United States

Central nervous system infection with the amoeba *Naegleria fowleri* leads to approximately 16 deaths each year in the US, with only four known survivors among all cases reported to date. Infection typically occurs following forceful inhalation of contaminated water during recreation, leading to rapidly progressing brain swelling and Primary Amoebic Meningoencephalitis (PAM). Despite the rarity of PAM, two recent serological surveys have reported >90% seropositivity to N. fowleri, indicating exposure to the amoeba is likely more common than previously thought. Therefore, the goal of this study is to understand N. fowleri seroprevalence among individuals on the University of South Carolina campus and risk factors for exposure. This study will recruit 500 individuals between the ages of 18-25 from across the country in April and September to donate a dried blood spot. Using these sample dried blood spots, we will do an ELISA to detect the presence of IgG and IgA antibodies to *N. fowleri.* Additionally, we will ask students questions about their outdoor recreational activities such as swimming, kayaking, and boating. The results of this study will increase our understanding of N. fowleri exposures among individuals in the USC community and how outdoor recreational activities may be associated with exposure.Further, understanding risk factors for exposure can help inform mitigation strategies to prevent future PAM cases.

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COMMUNITY KNOWLEDGE, ETHICS, AND ZOONOTIC SPILLOVER RISK: UNDERSTANDING BAT HUNTING PRACTICES IN BANGLADESH THROUGH A ONE HEALTH LENS

A. K. M. Dawlat Khan[1], Farhana Begum[2]

[1]Institute of Epidemiology Disease Control and Research, Dhaka, Bangladesh, [2]University of Dhaka, Dhaka, Bangladesh

Zoonotic risk is often framed through biomedical and ecological perspectives; however, community knowledge, livelihood realities, and ethical considerations play a critical role in shaping human-wildlife interactions. Bats are natural reservoirs of several emerging viruses, including Nipah, Hendra, Ebola, and Marburg, and are commonly hunted in Bangladesh, posing risks of deadly bat-borne zoonotic spillover to humans and animals. This study explores how spillover risk emerges within socio-economic and cultural contexts by examining local communities' understanding of bat-hunting practices. We conducted a qualitative ethnographic study in a bat-hunting community in Bangladesh from May 2017 to April 2018, including observations of bat-hunting events, in-depth interviews with 4 bat-hunters, and 20 individuals involved in bat meat processing and selling. The data was analyzed using a thematic analysis approach. The hunters hunted bats when there was no work to earn in

winter, as it provided an alternative source of income and lower-cost animal protein compared to domestic animal meats, which they could not afford at higher prices. They believed that bats don't transmit infections but are used as beneficial animals for treating several illnesses, like asthma, heart disease, and sexual vigor, by using bat meat and body parts. Bat processing commonly takes place in household spaces, where family members, including children, are exposed to bat blood and raw meat, and domestic animals such as dogs and cats consume bat offal. Hunters were also unaware of the Wildlife Act prohibiting hunting, capturing, or killing bats. The findings demonstrate that bat hunting is embedded in seasonal livelihood strategies and food insecurity rather than a simple lack of awareness about disease risk. Integrating community knowledge and ethical considerations should be implemented into One Health strategies through participatory risk communication, non-stigmatizing public health messaging, the Wildlife Protection Act, and alternative livelihood-sensitive interventions to reduce zoonotic spillover risks and support conservation.

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THE EFFECTS OF TWO HIGH RICE BRAN-BASED DIETS AND TWO LEVELS OF XZYME™ (AN EXOGENOUS ENZYME + PROBIOTIC FEED ADDITIVE) ON THE GROWTH PERFORMANCE AND CARCASS CHARACTERISTICS OF ALBINO RATS

Wallace Nii Lante Armah

Noguchi Memorial Institute for Medical Research, Accra, Ghana

The experiment was conducted to determine the effects of two high rice bran-based diets and 2 levels of XZYME™ (an exogenous enzyme + probiotic feed additive) on the growth performance and carcass characteristics of albino rats. The experimental diets contained two differing types of rice bran (RB) and each type had two levels of XZYME™ inclusion i.e. 250 and 500mg/Kg diet. The RB types were A (from Nobewam, more adulterated poor quality) and B (from Aboabo Zongo, less adulterated, good quality). There was a control (no RB) diet. Thirty albino rats with average initial live weight of 83.97 + 0.34g were randomly allocated to the 5 dietary treatments namely: T1 (Maize, Control), T2 (RB_{60} A+250mg Xzyme), T3 (RB_{60} A+500mg Xzyme), T4 (RB_{60} B+250mg Xzyme) and T5 (RB_{60} B+500mg Xzyme). There were 6 rats on each treatment, housed individually in plastic cages and each rat served as a replicate. Feed and water were provided *ad libitum* and their growth performance was monitored for 28 days. Carcass data were collected after slaughter. All the growth parameters showed significant differences among treatment means. Growth performance and carcass characteristics were better in T4 as well as the Control as compared to T2, T3 and T5. The feed cost/100g gain was lower in T4 but higher in treatments 2, 3 and 5 as well as the Control. From the results obtained, it was concluded that the addition of the 250mg of the additive to the type B rice bran-based diets led to a significant growth performance and carcass characteristics of the rats. Also the type B rice bran can partially replace maize at 60% in monogastric diets without any adverse effects on growth performance, except for an increase in feed intake and improved carcass characteristics. Such high quality RB-based diets with the additive were cheaper to formulate and feed.

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HOUSEHOLD ENVIRONMENTAL, STRUCTURAL, AND RODENT RELATED RISK FACTORS FOR ACUTE LASSA FEVER IN SIERRA LEONE

Foday Umaro Turay, John Schieffelin, Troy Moon

Tulane University, New Orleans, LA, United States

Lassa fever remains a major public health threat in Sierra Leone, but household-level factors associated with acute disease versus prior exposure are not fully understood. We analyzed household-level data from laboratory-confirmed Lassa fever cases and community households classified as IgG-negative or IgG-positive. Comparisons included cases versus controls (242 vs. 913), IgG-negative versus IgG-positive households (450 vs. 463), cases versus IgG-negative households, and cases versus IgG-positive households. Variables included district, housing materials, water source, sanitation-related proximity measures, and rodent access and contamination indicators. Univariable logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs). Cases were strongly geographically clustered, with most occurring in Kenema District. Across all case comparisons, the strongest and most consistent association was rodent holes observed on the exterior of the house: cases versus IgG-negative households (OR 24.77, 95% CI 15.97-38.42), cases versus IgG-positive households (OR 27.23, 95% CI 17.50-42.36), and cases versus controls (OR 25.56, 95% CI 16.98-38.48), all p=0.001. Rodent feces in the house and in sleeping areas were also strongly associated with case status. Housing characteristics, toilet proximity, and water source type differed significantly between cases and comparison groups. In contrast, rodent-related indicators were not strongly different between IgG-positive and IgG-negative households. Acute Lassa fever appears to be associated with a high-risk household environment characterized by rodent access, rodent contamination, and structural and sanitation-related features, while IgG seropositivity may reflect broader background exposure.

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ZOONOTIC TICK-BORNE PATHOGENS IN LIVESTOCK SLAUGHTERED AT A GHANAIAN ABATTOIR: A ONE HEALTH SURVEILLANCE STUDY

Margaret Addo[1], Seth Offei Addo[2], Patrick Kwasi Obuam[3], Christopher Nii Laryea Tawiah-Mensah[2], Richard Odoi-Teye Malm[2]

[1]SDA Hospital, Gbawe, Accra, Ghana, [2]Noguchi Memorial Institute for Medical Research, Accra, Ghana, [3]Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

Tick-borne zoonotic diseases are increasingly recognised as major contributors to febrile illnesses worldwide, but their epidemiology is poorly understood in many parts of Sub-Saharan Africa. Livestock and ticks play important roles in the persistence and spread of many diseases, particularly at the human-animal interface. Abattoirs serve as critical surveillance stations for detecting zoonotic infections in livestock populations. In this study, ticks and dry blood spots were collected from livestock slaughtered at the Kintampo abattoir and screened for tick-borne pathogens using PCR and Sanger sequencing. The data was analysed using R version 4.5.2. Of the 38 livestock sampled, including cattle (n=21, 55.3%), goats (n=12, 31.6%), and sheep (n=5, 13.1%), *Coxiella burnetii* was detected in 5.3% of the cattle samples. A total of 188 adult ticks were collected, with *Rhipicephalus microplus* (46.8%) as the predominant species. Out of the 59 tick pools, pathogen DNA was detected in 40 (67.8%). The pathogens identified were *Rickettsia africae* (49.2%), *C. burnetii* (39%), and *Rickettsia aeschlimannii* (10.2%). It was observed that *C. burnetii* (IRR= 5.00, 95%CI= 2.06 - 12.15, p<0.001) and *R. africae* (IRR= 2.34, 95%CI= 1.13 - 4.85, p=0.022) were more likely to be detected in male ticks. None of the samples was positive for Crimean-Congo Haemorrhagic Fever Virus. The identification of zoonotic pathogens *C. burnetii* and *Rickettsia* species reveals a significant but underappreciated zoonotic threat at Ghana's abattoirs. These findings underscore the importance of integrating One Health surveillance and enhancing diagnostics for tick-borne zoonoses within both veterinary and human health systems.

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GENOMIC EVIDENCE OF ZOONOTIC TRANSMISSION AND HYBRIDIZATION IN *STRONGYLOIDES* SPP IN BANGLADESH

Tilak Chandra Nath[1], Jamal Uddin Bhuiyan[1], Hamida Khanum[2], Mohammad Shafiul Alam[3]

[1]Sylhet Agricultural University, Sylhet, Bangladesh, [2]Dhaka University, Dhaka, Bangladesh, [3]icddr,b, Dhaka, Bangladesh

Strongyloides stercoralis is a globally distributed soil-transmitted helminth with a complex life cycle and recognized zoonotic potential. Molecular studies in Southeast Asia have identified genetically distinct "dog-only" and "human-dog" lineages; however, genomic data from South Asia remain limited. In contrast, *Strongyloides fuelleborni*, typically associated with non-

human primates, is rarely reported in humans in Asia. Understanding the genetic diversity and transmission dynamics of *Strongyloides* spp. is critical for assessing zoonotic risk and evolutionary processes. We conducted molecular and genomic analyses of *Strongyloides* spp. and hookworms collected from humans and a dog in two regions of Bangladesh. Species identification and lineage classification were performed using nuclear and mitochondrial markers, followed by phylogenetic analyses to assess genetic diversity and evidence of cross-species transmission and introgression. All hookworm isolates were identified as *Necator americanus*. Notably, multiple human infections with *S. fuelleborni* were detected, suggesting it may be underrecognized in Asia. Genomic analyses confirmed the presence of both "dog-only" and "human-dog" lineages of *S. stercoralis*, with no clear geographic structuring relative to Southeast Asian populations. A "dog-only" genotype was identified in a human host, supporting zoonotic transmission. Additionally, discordance between nuclear and mitochondrial genomes in canine isolates indicates mitochondrial introgression, suggesting rare hybridization events. These findings highlight previously underappreciated genetic diversity and transmission complexity of *Strongyloides* spp. in Bangladesh, with implications for zoonotic transmission and parasite evolution for parasite adaptation and the emergence of traits such as drug resistance.

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OUTBREAK OF LEPTOSPIROSIS AMONG FARMERS IN DAN AND PANGSHA VILLAGES, NOKLAK DISTRICT, NAGALAND, INDIA, SEPTEMBER 2024

Takujungla Jamir[1], Nyanthung Kikon[2], John Kemp[2], Limasenla Lemtur[3], Impokchala Jamir[2], Urshela Punanamai[2], Tsukjemsangla Jamir[4], Shiela Longkumer[5], Longri Kichu[1], Mehnaz (aka Geeta) Parvez[6]

[1]Nagaland University, Kohima, India, [2]Integrated Disease Surveillance Programme, Directorate of Health & Family Welfare, Government of Nagaland, Kohima, India, [3]Integrated Disease Surveillance, Directorate of Health & Family Welfare, Government of Nagaland, Kohima, India, [4]Integrated Disease Surveillance Programme, directorate of Health & Family Welfare, Government of Nagaland, Kohima, India, [5]World Health Organisation, Nagaland, India, Kohima, India, [6]St. Catherine University, Saint Paul, MN, United States

Globally, about 1 million leptospirosis cases in people occur annually, with nearly 60,000 deaths. In India, burden of human leptospirosis is likely underestimated because of limited laboratory capacity and due to the overlapping symptoms with other flu-like illnesses. District Surveillance Unit Noklak reported 108 flu-like cases at Dan and Pangsha villages to State Surveillance Unit Nagaland on August 26, 2024 following incessant rains. Our team started the investigation on August 27, 2024 to describe the epidemiological characteristics of outbreak and to determine the associated risk factors. We defined a case of leptospirosis as sudden onset of fever with two or more signs and symptoms occurring in a resident of Dan and Pangsha villages from August 16, 2024 to September 20, 2024. We did a house to house survey for cases and conducted a retrospective cohort study on people with flu-like illness. We observed exposure to animals and associated risk factors. We collected 14 serum samples for testing Leptospira IgM antibodies and one water sample. We identified 138 cases with an attack rate of 8%. There were no hospitalizations and no deaths. Attack rate was highest in the age group of 46-60 years (13%) followed by 61-83 years (12%) and 6-15 years (8%). Females (53%) were more affected. Among the identified cohort of 138 people, 48 did not have fever but flu-like symptoms. Major presenting symptom was fever (65%) followed by cough (59%) and headache (54%). Risk of leptospirosis was 3 times (95% CI=1.6-5.8) among those who did not wear protective gears and 2.3 times (95% CI= 1.6-3.3) among paddy/cardamom farmers. Of the 14 serum samples, six were positive for IgM antibodies for leptospirosis. Water sample was found to be within permissible limits as per IS:10500:12. An outbreak of leptospirosis occurred following higher than usual rainfall at Dan and Pangsha villages having strong association with not wearing protective gear. We recommended IEC activities regarding protective clothing, proper hygiene practices, avoidance of contact with contaminated water and timely collection of appropriate samples for testing.

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PURSUING MALARIA REDUCTION IN NORTHERN ZAMBIA WITH A ONE HEALTH LENS

Shelby M. Cagle[1], Margaret V. Becker[1], Renee LMN Ali[1], Mbanga Muleba[2], Westone P. Hamwata[2], James Lupiya[2], Hunter Chilusu[2], Douglas E. Norris[1]

[1]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [2]National Health Research & Training Institute, Ndola, Zambia

Nchelenge District in northern Zambia experiences high, sustained, year-round malaria transmission. Control and elimination of malaria is challenged by factors including but not limited to ecosystem complexity, human behaviors, insecticide resistance, diagnostic gaps, and access to medical care. While previous studies have considered human behavioral characteristics, such as bed net usage, our study seeks to implement a One Health lens to further investigate how anthropogenic and ecological complexities may facilitate exposure to malaria vectors in this region and to explore household-level characteristics that contribute to transmission risk. Previous studies show Anopheles funestus and An. gambiae are predominant vectors in Nchelenge; however, the breeding sites of these mosquitoes in this region and the abundance per household are unknown. Bodies of water and seasonal rains are hypothesized to drive mosquito abundance near community members and their livestock. Adult mosquito trapping inside and outside of households, and larval collections at identified breeding sites will be conducted monthly to determine mosquito species abundance and diversity. Concordantly, household members will complete monthly surveys to capture self-reported disease incidence, herd health, agricultural and fishing practices, household structure composition (i.e. roofing materials, windows, location), and behaviors of community members. Data will be analyzed to determine ecological changes, impacts on fishing and agriculture, mosquito bite risk behaviors, house construction, and community practices contributing to sustained malaria rates in Nchelenge District. Findings will be used to guide efficacious, community-based vector control strategies such as house screening and interventions at community gathering places. Science communication and education strategies for malaria reduction will be developed accordingly and alongside community partners.

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IMMUNOSUPPRESSION DOES NOT AFFECT ECHINOCOCCAL CYST VIABILITY, AS PROVEN BY IMMUNOBLOTTING TEST: A CASE REPORT IN A LUNG TRANSPLANT PATIENT

Raffaella Lissandrin[1], Ambra Vola[2], Domenica Federica Briganti[2], Sofia Frattola[3], Armanda Leka[3], Guido Baietto[2], Carlo Pellegrini[2], Elena Seminari[2], Enrico M. Brunetti[4]

[1]Foundation IRCCS Policlinico San Matteo, WHO Collaborating Centre for Clinical management of Cystic Echinococcosis, Pavia, Italy, Pavia, Italy, [2]Foundation IRCCS Policlinico San Matteo, Pavia, Italy, [3]University of Pavia, Pavia, Italy, [4]University of Pavia, San Matteo Hospital Foundation, WHO Collaborating Centre for Clinical management of Cystic Echinococcosis, Pavia, Italy

A 61-year-old Italian woman was diagnosed with an inactive (CE4) echinococcal cyst, in the VII hepatic segment in 2020. The cyst measured 50 x 50 mm, and being inactive and asymptomatic, was managed conservatively ("Watch and Wait") as per WHO Guidelines. Serology for *E. granulosus* (ELISA) was negative. In September 2025, the patient underwent bilateral lung transplant for chronic obstructive pulmonary disease. Induction with basiliximab, steroids and a following treatment with tacrolimus and mycophenolic acid as anti-rejection treatment was started. Serology for *E. granulosus* [(ELISA, rapid test (RT) and immunoblotting (IB)] was negative. No treatment, including albendazole, was administered given the inactive CE4 stage. Serology with ELISA, RT, and IB remained

negative at 0, 1, 3 and 6 months after the transplant. The cyst remained inactive at US in all the evaluations. This supports the hypothesis that, unlike with Alveolar echinococcosis (AE), immunosuppression has no effect on viability of *E. granulosus* cysts. This has important clinical consequences. The American Society of Transplantation suggests to treat patients before transplant, and for adequate candidates, recommends surgical removal with pre-and post-surgical albendazole. However, literature supporting this recommendation is almost non-existent. In 1993, Sobrino JM et al described a patient with one hepatic CE3b cyst who underwent heart transplant for ischemic cardiomyopathy, but only information on the unchanged size of the cyst 14 months post-op was given. The stage-specific approach to treatment of liver CE advocated by the WHO guidelines is important to avoid unnecessary procedures. This might well apply to patients that face immunosuppression due to solid organ transplant. We suggest that clinicians dealing with these patients consult established referral centers for CE before embarking on debatable management choices.

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USE OF A SIMPLIFIED URINE DNA EXTRACTION WORKFLOW FOR A NON-INVASIVE NEUROCYSTICERCOSIS DIAGNOSIS

Luz M. Toribio[1], Javier A. Bustos[2], Hector H. Garcia[1]

[1]Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Universidad Peruana Cayetano Heredia, LIMA, Peru

Neurocysticercosis (NCC), a neglected parasitic infection of the central nervous system caused by *Taenia solium*, remains difficult to diagnose in endemic settings. Although urine-based molecular detection offers a non-invasive approach, current DNA extraction methods require laboratory infrastructure. We evaluated a simplified DNA extraction workflow (ProtonDx-SmartLid) and compared its performance to the standard spin-column protocol for urine-based diagnosis. Urine samples from individuals with NCC (confirmed by imaging) and controls (n=10) were selected, and 4 mL per sample was processed by silica column and SmartLid protocol, which uses magnetic bead capture. Extracted cfDNA was quantified and *T solium* cfDNA detected by PCR targeting the pTsol-9 gene, with amplicons visualized in 2% agarose gel. Quantification of total cfDNA was significantly greater using the bead-based method in all samples (cfDNA varying from 22.54-114.4 for bead-method versus 2.54-48.90 ng/ml for spin column-method, p<0.028). The bead-based method detected *T. solium* DNA in 88.9% (8/9) of samples compared to 77.8% (7/9) using spin-column extraction, with comparable or improved band clarity on agarose gel. The negative control remained PCR-negative with both extraction methods. In addition to analytical performance, operational characteristics were assessed. The simplified workflow reduced the number of procedural steps from 14 to 6, decreased hands-on time in ~48%, and required minimal equipment compared to the spin-column method (no centrifugation). Usability was qualitatively assessed, with technicians reporting improved ease of use and rapid adoption following a single demonstration. Although based on a small sample size, these findings suggest that simplified extraction workflow maintained diagnostic performance while substantially reducing processing time and complexity, enabling rapid training and implementation in low-resource settings. Further studies will focus larger evaluation of samples supporting the feasibility of decentralized molecular detection of NCC and could improve access to NCC detection in endemic regions.

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ANALYSIS OF THE GUT MICROBIOTA IN PATIENTS WITH TAENIASIS BEFORE AND AFTER ANTHELMINTIC TREATMENT USING OXFORD NANOPORE SEQUENCING

Mishell Landa Flores, Fresie Denisse Maciel Carpio, Javier Bustos Palomino, Mirko Zimic Peralta, Omar Romero Rodríguez, Hector Garcia Lescano

Universidad Peruana Cayetano Heredia, Lima, Peru

Taenia solium taeniasis remains a relevant public health problem in endemic areas. Although intestinal infection is often asymptomatic, human carriers play a central role in parasite transmission. Because the gut microbiota contributes to immune modulation and host homeostasis, its characterization may provide insight into host-parasite interactions and mechanisms involved in infection establishment and persistence. We evaluated whether *T. solium* infection is associated with alterations in gut microbiota composition and diversity and whether these profiles differ between taeniasis patients and non-taeniasic individuals and change after anthelminthic treatment. We conducted an observational analytical study using fecal samples from individuals with taeniasis collected before and after niclosamide or praziquantel treatment and from matched non-taeniasic controls. Metagenomic DNA was sequenced with Oxford Nanopore technology. We analyzed 23 pre-treatment samples, 25 post-treatment samples, and 43 controls. Active taeniasis was associated with enrichment of *Prevotella* spp. and related taxa, including *Sodaliphilus* and *Pseudoprevotella*, whereas controls showed greater representation of *Bifidobacterium bifidum* and *Bacteroides finegoldii*. LEfSe identified discriminant taxa across groups, with log10 LDA effect sizes generally ranging from ~2 to 4. After treatment, *Prevotella*-related markers decreased and taxa such as *Butyrivibrio crossotus* and *Bacteroides* emerged, suggesting partial reversal of the dysbiotic profile. Active infection also showed a trend toward lower proportions of butyrate-producing taxa, including *Roseburia*, *Butyrivibrio*, and *Coprococcus*. These findings suggest the presence of a microbial signature associated with active *T. solium* taeniasis that may be partially attenuated after anthelminthic treatment.

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GIANT ECHINOCOCCAL CYST OF THE PELVIS WITH MUSCULOSKELETAL INVOLVEMENT MANAGED WITH MINIMALLY INVASIVE DRAINAGE

Sofia Frattola[1], Gian Luca D'Alessandro[2], Armanda Leka[1], Ambra Vola[3], Islam Abumalouh[3], Raffaella Lissandrin[3], Fabrizio Cuzzocrea[3], Federico Grassi[1], Enrico Brunetti[1]

[1]University of Pavia, Pavia, Italy, [2]ASST Melegnano Martesana, Pavia, Italy, [3]San Matteo Hospital Foundation, Pavia, Italy

Cystic echinococcosis is a chronic parasitic infection characterized by cyst formation, most commonly in the liver and lungs. Symptoms usually arise from complications (e.g., superinfection or rupture) or compression of adjacent structures. Extra-abdominal involvement may lead to complex, disabling presentations poorly responsive to medical therapy. Most patients undergo non-resolutive surgeries, facing chronic disability. Musculoskeletal involvement is rare, even in endemic areas, and its prevalence is unclear. In the absence of established guidelines and with literature largely limited to case reports, management relies heavily on clinical experience. A 67-year-old Moroccan man sought medical attention for the enlarging groin mass which he had been carrying for six years. He was initially admitted to the oncology unit on suspicion of sarcoma and later referred to an orthopedic hospital for surgical removal. A preoperative infectious disease consultation raised suspicion of cystic echinococcosis, and he was referred to our hospital. He was diagnosed with a large (15 cm maximum) CE3b cyst extending from the right iliopsoas muscle to the lower third of the thigh. Radical surgery was excluded due to disease extent and high operative risk in the absence of neurological deficits. Albendazole was started but discontinued after two months for hepatic toxicity and replaced with well-tolerated mebendazole. Annual ultrasound follow-up showed good clinical response defined by reduction in number of daughter cysts. Three years

later, gait progressively worsened with recurrent falls. MRI demonstrated bladder compression and neurovascular displacement. The patient declined percutaneous drainage and continued medical therapy. Over the next four years, functional impairment increased and the mass enlarged, as confirmed by ultrasound and MRI. The patient eventually underwent uncomplicated percutaneous debulking, after which regular ultrasound monitoring of the cyst was resumed.

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WHEN COLONOSCOPIES GET WORMY: AN UNEXPECTED CASE SERIES

Andy G. Bae[1], Elena M. Crecelius[2], Kimera Joseph[3], Patrick W. Hickey[1], Alison M. Helfrich[1]

[1]Uniformed Services University of the Health Sciences, Bethesda, MD, United States, [2]Department of Pediatrics, Walter Reed National Military Medical Center, Bethesda, MD, United States, [3]Department of Pediatrics, Naval Medical Center Portsmouth, Portsmouth, VA, United States

Soil-transmitted helminths (STH) infect approximately a quarter of the global population with an incidence of 3-5 per 100,000 people within the U.S. Military Health System (MHS). These parasitic nematodes infect the gastrointestinal tract causing abdominal pain, distension, malabsorption, and anemia. Prior reports have noted cases of infections incidentally diagnosed via colonoscopy. U.S. military service members are a unique population due to their increased risk of exposure to STH on deployments and overseas assignments. From a cohort of 206 cases of STH in the MHS, we identified nine cases diagnosed via colonoscopy: two cases of trichuriasis and seven cases of hookworm. This case series describes the epidemiology, clinical features, travel history, and findings that may improve screening strategies. Ages ranged from 11 to 66 years, with one pediatric case. Three cases occurred in service members and six cases occurred in military family members. The majority of cases were females. Six cases reported previous travel to and/or habitation in a STH-endemic country: four reported the Philippines as their country of birth (two of whom reported subsequent return travel to the Philippines), one had a prior military assignment (duty station) in Guam, and one had a military deployment to Afghanistan. Only two patients had symptoms of a STH intestinal infection (fatigue, dizziness, and/or hematochezia), while 78% were asymptomatic, diagnosed incidentally on routine colonoscopy performed for other indications. Of the five with a blood count obtained, only two exhibited peripheral eosinophilia >500 cells/µL. This is the first study to present STH infections diagnosed via colonoscopy in the MHS. Travel to and/or habitation in an STH-endemic country appear to be significant risk factors. Our identification of asymptomatic STH-infections as incidental findings on colonoscopy suggests a potentially underestimated disease burden. Utilization of colonoscopy for screening is inefficient as a diagnostic measure for STH-infections, necessitating a move towards proactive, risk-based screening strategies and optimized diagnostic pathways.

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HOOKWORM INFECTION IN BEPOSO, GHANA: RESULTS OF A LONGITUDINAL SEROEPIDEMIOLOGIC ANALYSIS

Savanna Randi[1], Irene Owusu Donkor[2], Dickson Osabutey[2], Jeffrey Gabriel Sumboh[2], Christopher Dorcoo[2], Amanda Lamptey[2], Isaac Quaye[2], Charlene Wulff[2], Grace Odoom[2], Rahmat Yusif Ismail[2], Kojo Mensah Sedzro[2], Francis Appiah-Twum[2], Miranda Josiah[2], Prince Zowonu Horlortu[2], Doreen Plange[2], Joseph Quartey[2], Mark Tetteh-Tsifoanya[2], Philip Mensah[2], Daniel Odumang[2], Ruth Ajaloko[2], Taylor Clarkson[3], Angelica Daakyire[2], Elizabeth Obeng-Aboagye[2], Portia Owusua Aniapam[2], Elvis Suatey Lomotey[2], Ayush Iyer[1], Samantha Gillis[1], Christina Paraggio[1], Martina Wade[1], Rick Bungiro[1], Veronika Shabanova[1], Anita Ghansah[2], Michael Cappello[1]

[1]Yale School of Public Health, New Haven, CT, United States, [2]Noguchi Memorial Institute for Medical Research, Accra, Ghana, [3]University of Nebraska Medical Center, Omaha, NE, United States

Despite the significant public health challenge posed by soil-transmitted helminth (STH) infections in rural, resource-limited areas across sub-Saharan Africa, transmission dynamics and host immune responses remain poorly defined. In 2022, a 5-year longitudinal cohort study was initiated in Beposo, Ghana, to define hookworm epidemiology and response to repeated deworming. At baseline, 1,047 subjects (aged 3-100 years; 51% male) were enrolled and completed a questionnaire to assess demographic, behavioral, and socioeconomic risk factors. Fecal samples (n=996) were screened for hookworm eggs using Kato-Katz microscopy. Hookworm-infected subjects (n=285) were selected for further study, along with an age-matched group of uninfected subjects (n=370). Among 443 participants with complete longitudinal follow-up, hookworm prevalence decreased from 43.6% (193/443) at baseline to 30.9% (137/443) in 2023, 23.0% (102/443) in 2024, and finally 11.7% (52/443) in 2025. Deworming resulted in treatment cure rates of 58.4% in 2023, 49% in 2024, and 72.3% in 2025. Geospatial analyses of hookworm burden also indicate a progressive decline in infection intensity across the study area. Baseline serological analysis of the study cohort identified IgG antibody reactivity to *N. americanus* adult worm excretory/secretory proteins (AUC of 0.75), with significant discrimination between hookworm-positive and negative individuals. Over the 4-year study period, IgG antibody responses declined across both infected and uninfected groups, suggesting a community-wide reduction in exposure and possibly transmission. A follow-up study in 2026 will compare hookworm prevalence and seroreactivity between cohort participants and community members who were not previously enrolled. As countries reevaluate mass drug administration strategies, there is an urgent need to quantify both the direct and community-level (indirect) effects of targeted interventions. These findings highlight the value of integrating longitudinal epidemiological, geospatial, and serological data to inform adaptive STH control strategies in endemic areas.

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AN INVESTIGATION OF SOIL-TRANSMITTED HELMINTHS IN FECAL SLUDGE TREATMENT PLANTS (FSTP) AND ADJACENT SOIL: ROHINGYA CAMP, COX'S BAZAR, BANGLADESH

Zahid Hayat Mahmud[1], Nafisa Nawal Labiba[2], Md. Sakib Hossain[1], Hajbiur Rahman[1], Rifaquat Ahmed[1], Md. Foysal Abedin[1], Mohammad Atique Ul Alam[1], Ashrin Haque[1], Mohammad Rafiqul Islam[1], Md. Shafiqul Islam[1]

[1]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [2]BRAC University, Dhaka, Bangladesh

Soil-transmitted helminth (STH) infections remain a major public health concern, affecting approximately 2 billion individuals with either one or multiple STH infections. These are ubiquitous in the tropical and subtropical regions and the infection results in helminthiasis, which is listed among the top 13 neglected tropical diseases (NTD). STH infections primarily target the impoverished and marginalized communities for instance densely

populated humanitarian camps and slums. The lack of personal and communal hygiene turns these setups into a breeding ground for STH. There are limited data on the presence of STH in wastewater and soil in humanitarian locations like the Rohingya camps. This study, therefore, focuses on the prevalence and species distribution of STH in inlet and outlet wastewater, as well as adjacent soil samples from five different locations of fecal sludge treatment plants (FSTPs) over the period of one year (August, 2024- July, 2025). The FSTPs included anaerobic baffled reactor (ABR), anaerobic treatment plant, aerobic treatment plant, decentralized wastewater treatment system (DEWATS) and mega FSTP. Among the 120 inlet and outlet samples and 60 soil samples, STH egg was consistently detected in both soil and inlet sludge. However, the treated outlets harbored no egg, except DEWATS facility. The most predominant STH was *Ascaris* spp. (~85%), followed by *Trichuris* spp. (~5%) and other species (~10%) in both matrices. Although the FSTPs outlets were mostly egg free due to the treatment, the high egg count in soil jeopardizes the communal health. Walking barefoot, children playing in soil, eating contaminated crops and vegetables and open defecation can lead to a higher risk of STH infection for the Rohingya community. Strategies like WASH interventions, general public awareness, mass drug administration and routine clinical surveillance are effective to control the burden of STH infection and lower the risk of further transmission in humanitarian settings.

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CHILD-LED, RIGHTS-BASED EDUTAINMENT FOR STH & SCHISTOSOMIASIS PREVENTION IN KENYA

Faith Kathambi Mutegi

Amref Heath Africa, Nairobi, Kenya

In western Kenya, soil-transmitted helminthiasis and schistosomiasis persist among school-aged children despite mass drug administration, as conventional messaging excludes local languages and marginalized groups. We hypothesized that a child-led, rights-based edutainment model would increase prevention knowledge and community ownership. A six-episode series, 'Sikicho's Adventures,' was co-created with Akili Kids, Project Hand Up, and communities across four high-burden counties. Local child champions (ages 8-14) demonstrated prevention behaviors in five mother tongues (Maragoli, Lubukusu, Luhya, Luo, Kisa); one episode featured a mother with a disability. The series was broadcast ≥4 times weekly on national TV during school holidays and in schools via health clubs, reaching 8.16 million children and 5.79 million adults weekly across 38 counties. SMS feedback from 1,538 viewers showed 53% demonstrated accurate prevention knowledge, with 31% showing comprehensive understanding. Unsolicited child messages reflected strong emotional connection and content ownership. This child-led, mother-tongue, inclusive design strengthened NTD prevention, advanced community ownership, and reached marginalized groups, contributing to equitable public health and universal health coverage goals.

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THE PREVALENCE OF SCHISTOSOMIASIS HAEMATOBIUM CAUSING FEMALE GENITAL SCHISTOSOMIASIS AMONG WOMEN ATTENDING ANTENNAL CARE 16-39 YEARS AT G.W. HARLEY HOSPITAL, NIMBA COUNTY: A DESK REVIEW OF CASES FROM LABORATORY EXAMINATION FOR URINALYSIS AND HEMASTIX 2021-2025

Comfort Lavela Allison[1], Emmanuel Sonkalay[2], Theodosia Welma Knight[3]

[1]Phebe School of Nursing and Midwifery, Gbarnga, Bong County, Liberia, [2]Nimba University, Sanniquellie, Liberia, [3]Beijing Normal University, Beijing, China

Schistosomiasis is a disease of poverty that affects poor and rural communities which leads to chronic ill-health. Infection is acquired when people who are engage domestic work in infested water, such as washing clothes are also at risk and can developed female genital schistosomiasis. particularly agricultural and fishing populations. Female genital schistosomiasis (FGS) is a serious public health issue which affects women and girls living in schistosomiasis (SCH) endemic areas. About 56 million women and girls are estimated to be affected by FGS in Sub-Saharan Africa (WHO, 2029). This condition occurred due to the repeatedly exposure of women and girls to Schistosoma parasites. The schistosome eggs become trapped in the genital tissue leading to inflammation and damage to the tissue. A delay in seeking and receiving treatment for FGS symptoms can lead to the worsen clinical outcome that may be associated with conditions such as cervical cancer, infertility miscarriages, ectopic pregnancies. This condition can increase in infant and maternal death in areas were the prevalence of Schistosomiasis. Haematobium is high. It can also lead to severe psycho-social and economic consequence where the social status of women is often associated with fertility and reproductive capacity making women to lose their relationship due to health implication such as infertility and stigma associated with FGS. The prevalence of SCH in Nimba county is about 43.32%, and Sanniquellie District where the study was conducted has the prevalence of 42.8% based on the mapping conducted from 2013-2015 in Liberia. The aims of this study were to identify pregnant women with S. haematobium who attended antennal care at the at the G.W. Harley Maternity Center. To know the total number of pregnant women affected with S. haematobium from 2021-2025. The method used was a desk review of records of all women who attended antennal care and had urinalysis test and hemastix for blood in the urine for S. haematobium from 2021-2025. A total of 294 women were tested for S. Haematobium and 221 were positive. This study will inform the Ministry of Health the step to take in managing FGS in Liberia

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THE PREVALENCE OF INTESTINAL PARASITES IN BASIC SCHOOL CHILDREN AT ASOKORE MAMPONG MUNICIPALITY, GHANA

Selassie Adjoa Bruku[1], John Asiedu Larbi[2], Sandra Siawbea Gyarteng Mensah[2], Evans Anokye Kumi[2]

[1]Noguchi Memorial Institute for Medical Research, Accra, Ghana, [2]Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

Soil transmitted helminths pose a major threat to children especially in tropical areas of East Asia, sub-Saharan Africa and South America and known to infect children with over 24% (more than 1.5 billion) of the world population being infected. Parasitic infections in school children are widespread especially in tropical areas with its relative morbidity and mortality. The study aimed to identify intestinal parasitic infections and determine the associated risk factors among school children at Asokore Mampong Municipality in Ghana. Stool samples were randomly collected from a hundred and twenty school children and analysed using direct wet mount preparation and formol ether concentration methods for the identification of protozoan trophozoites and cysts, helminth ova and larva. The protozoan parasites identified included *Gardia lamblia* and *Entamoeba histolytica* while the helminths identified were Hookworm, *Ascaris lumbricoides*, *Strongyloides stercoralis, Trichuris trichiura* and *Schistosoma mansoni*. Out of the total study participants (120), 28% recorded no infection; 26.7% had single parasitic infection whiles 3.3% had two parasite species. Multiple parasitism was recorded in 11.7% of the school children. *A. lumbricoides* was of the highest prevalence of 59.7% followed by *S. stercoralis* with a prevalence of 43.3%. Hookworm and *E. histolytica* with a prevalence of 10% each. *S. mansoni*, *G. lamblia*, and *Enterobius vermicularis* each recorded a prevalence of below 10%. *S. stercoralis* had a mean intensity of 18.85 Eggs per Gram (EPG). Males recorded a higher prevalence of parasitic infections than females. Intestinal parasitic infections were prevalent among the basic school children within this community therefore appropriate control measures such as regular deworming must adopted to counteract the rate of infection.

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SURVEY OF INTERMEDIATE HOSTS OF *ANGIOSTRONGYLUS CANTONENSIS* IN GUAM

Eugene W. Liu[1], Daniel E. Urbano[2], Anna Ursales[3], Richard Singh[4], George C. Fiedler[5]

[1]Loma Linda University School of Medicine, Loma Linda, CA, United States, [2]University of Guam, Mangilao, GU, United States, [3]Loma Linda University, Loma Linda, CA, United States, [4]Western Pacific Tropical Research Center, University of Guam, Mangilao, GU, United States, [5]College of Natural & Applied Sciences, University of Guam, Mangilao, GU, United States

Angiostrongyliasis is a parasitic disease that historically has been present in Guam. Human cases have been reported in Guam and as well as its causative agent, *Angiostrongylus cantonensis*, in *Lissachatina fulica* snails (giant African land snail) in the 1960s. However, it remains unknown if *A. cantonensis* remains present on this Pacific Island, particularly given the inadvertent introduction of *Boiga irregularis* (brown tree snake), which is known to prey on rats that serve as definitive hosts for *A. cantonensis*. It is also unclear if *A. cantonensis* is present in recently introduced gastropod hosts such as *Satsuma succincta* or *Veronicella cubensis* (Cuban slug). Presence of *A. cantonensis* in these introduced hosts could lead to outbreaks of disease. The ongoing presence of *A. cantonensis* in Guam would be of public health concern given the risks of contamination of the food supply and the development of outbreaks of angiostrongyliasis. To assess the risk of angiostrongyliasis to the population of Guam, we conducted a survey of suspected intermediate hosts of *A. cantonensis* using microbiologic and molecular methods. Thus far, we have surveyed 13 convenience sites in central and southern Guam where gastropods are known to be present, identifying two sites with *L. fulica*, four with *Pomacea canaliculate* (apple snails), five with Satsuma spp., and three with *Veronicella cubensis*. Processing thus far of gastropods using a Baerman funnel has demonstrated nematodes with morphology consistent with *Angiostrongylus* spp in one isolate of *L. fulica*. No nematodes have been isolated thus far in any of the other species collected. Pending qPCR for *A. cantonensis (*AcanR3990) on these nematodes may help to confirm the presence of *A. cantonensis* in this known intermediate host. Our results thus far suggest that *A. cantonensis* remains endemic to Guam, and may pose a risk to the local food supply.

6398

BURDEN OF *TOXOCARA* SPP. INFECTIONS IN SCHOOL CHILDREN IN THE ASHANTI AND EASTERN REGIONS, GHANA

Kweku Gyesi Hope[1], Quinsta Adomah[2], Bismark Acheampong[2], Beatrice Damptey[2]

[1]University of Skills Training and Entrepreneurial Development, 00233, Ghana, [2]University of Skills Training and Entrepreneurial Development, Kumasi, Ghana

Toxocariasis is a neglected parasitic disease with substantial socio-economic consequences, particularly in low-resource communities, and it is estimated to affect millions of children worldwide. This study investigated the prevalence and risk factors of *Toxocara* spp. infections in school children in the Ashanti and Eastern regions of Ghana. A cross-sectional survey was conducted among 224 schoolchildren from the Ashanti (118) and Eastern (106) regions. Approximately 2 mL of venous blood was collected by certified phlebotomists and centrifuged using an Eppendorf 5430R refrigerated centrifuge to obtain serum, buffy coat, and red cells. The serum was then analyzed by ELISA for anti-*Toxocara* IgG antibodies using TES-Ag.The findings revealed a rate of positivity of human toxocariasis of 21.4% (48/224), with significant regional variation. School children 5-12 years recorded the highest positivity rate, at 12.5% (28 out of 224 individuals tested), followed by those in the 13-16-year category (5.4%; 12/224). Regular contact with dogs (OR = 2.3, $p = 0.01$), frequent soil exposure (OR = 3.0, $p = 0.02$), poor nutritional status (OR = 2.4, $p < 0.01$), and infrequent deworming (OR = 3.0, $p < 0.01$) were identified as significant risk factors. Infected school children exhibited elevated anaemia, IgE levels and eosinophil counts. These results emphasize the importance of adopting integrated human–animal health strategies to effectively reduce the transmission of *Toxocara* spp. in endemic areas.

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PREVALENCE AND DISTRIBUTION OF INTESTINAL PARASITIC INFECTIONS IN CATTLE IN THE SEKYERE TRADITIONAL AREA, GHANA

Helina Osei Tutu[1], Kofi Agyapong Addo[2], Emmanuel Baafi[2]

[1]Abuakwa Government Polyclinic, Kumasi, Ghana, [2]University of Skills Training and Entrepreneurial Development, Kumasi, Ghana

Cattle production plays a crucial role in the agricultural economy of most nations; however, their parasitic infestations remain a major constraint to optimal production. This study was therefore aimed at determining the diversity of intestinal parasites and their zoonotic potential in Cattle from Sekyeredumase, Amanten and Atebubu. The study employed a cross-sectional design, in which stool samples were collected directly from the rectum of 250 Cattle: Sekyeredumase (75), Amanten (77) and Atebubu (98). To enhance detection, the flotation technique was used to concentrate the parasitic stages present in the samples. The concentrates were microscopically examined for the identification of parasite eggs, cysts and larvae. An overall prevalence of 71.2% (178/250) of intestinal parasites was recorded in cattle in the study area. The highest prevalence of intestinal parasites was recorded in Cattle from Amanten (28.4%; 71/250), followed by Cattle at Atebubu (26%; 65/250), while the lowest infection rate was found in Sekeyeredumase (16.2%; 42/250). Trematodes (38.4%), nematodes (27.2%), and protozoa (16.8%) were the three most common parasitic groups identified. About 18.8% of the intestinal parasite-infected cattle were observed to be carrying multiple parasites. *Fasciola* spp (18.2%) was the dominant trematode species observed; *Oesophagostomum* spp (12.4%) was the dominant nematode observed, while *Eimeria* spp. (8.7%) was the dominant protozoan observed. Cattle in the study area remain burdened by intestinal parasites. This underscores the need to strengthen the use of properly processed and hygienic feed, as well as improved feeding practices, to prevent parasite transmission and reduce infection rates.

6400

PREVALENCE, SPECIES DISTRIBUTION, AND ZOONOTIC POTENTIAL OF GASTROINTESTINAL PARASITES IN DOGS IN BOSOMTWE DISTRICT, GHANA

Frank Essem, Kofi Agyapong Addo, Samuel Ofori Ayetibo, Papa Kofi Amissah-Reynolds

University of Skills Trianing and Entrepreneurial Development, Kumasi, Ghana

Gastrointestinal parasites are major enteric pathogens and a leading cause of morbidity and mortality in dogs. These infections not only compromise canine health, affecting nutrition, growth, and overall wellbeing, but also pose significant zoonotic risks, with the potential for transmission to humans. This study assessed the prevalence of gastrointestinal parasites in dogs and owners' preventive practices in Bosomtwe, Ghana.A total of 194 dogs were included in the study. Faecal samples were collected directly from the rectum using sterile swab sticks. The swabs were then washed in flotation solution and examined microscopically for the presence of gastrointestinal parasite eggs, cysts, and larvae.Overall, 46.9% of the dogs were infected with at least one gastrointestinal parasite. Age-specific analysis showed that puppies had the highest infection rate (21.6%), followed by young dogs (14.9%) and adults (10.3%). Based on usage, hunting dogs recorded a higher infection rate (28.4%) compared to companion dogs (7.2%). The most frequently identified parasites were hookworms (n = 39), followed by *Toxocara canis* (n = 26), *Dipylidium caninum* (n = 14), and *Trichuris vulpis* (n = 8). Polyparasitism was observed in 66 dogs. Notably, the majority (72.5%) of hunting dogs had not been dewormed by their owners, which may have contributed to the higher infection rates observed in this group.The study demonstrates a high

prevalence of gastrointestinal parasitic infections in dogs, including several zoonotic species with the potential for transmission to humans. These findings underscore the significant public health implications, particularly in communities where close human–animal interactions and inadequate deworming practices are common.

6401

WILDLIFE RESERVOIRS OF *STRONGYLOIDES* SPP. AND *ASCARIS* SPP. AND THEIR ONE HEALTH IMPLICATIONS

Joanita Asirifi Yeboah[1], Winnifred Offih-Kyei[1], Caleb Kobina Danso-Coffie[2], Rhoda Yeboah[2], Daniel Oduro[3]

[1]Noguchi Memorial Institute for Medical Reserach, Accra, Ghana, [2]Noguchi Memorial Institute for Medical Research, Accra, Ghana, [3]UNIVERSITY OF GHANA, Accra, Ghana

Wild animals act as reservoirs of soil-transmitted helminths, sustaining ecological transmission and posing zoonotic risks. This study investigated *Strongyloides* spp. and *Ascaris* spp. in wildlife to evaluate their role at the human-wildlife interface. Fecal samples were collected from 50 wild animals comprising of rodents (n = 37), ungulates (n = 10), and wild cats (n = 3) at a major bushmeat market in Kumasi, Ghana, representing diverse wildlife hosts with different ecological behaviors that influence parasite exposure and transmission. Coprological and PCR analyses revealed high prevalence across wild animal groups. *Strongyloides* spp. were detected in 59.5% of rodents, 66.7% of cats, and 90.0% of ungulates, while *Ascaris* spp. occurred in 56.8% of rodents, 100% of cats, and 60.0% of ungulates. These parasites are transmitted through soil-mediated pathways, including ingestion of embryonated eggs or skin penetration by infective larvae, allowing persistence in warm, humid environments. The high prevalence across host taxa suggests ongoing transmission within wildlife populations and environmental contamination. Although the bushmeat market represents a direct point of human exposure through handling and trade, the broader concern is the sustained circulation of helminths within wildlife and surrounding ecosystems. These findings highlight the need for integrated One Health surveillance addressing wildlife reservoirs, environmental sanitation, and human behavioral risks to reduce spillover potential.

6402

MOLECULAR CHARACTERIZATION AND RISK MODELLING OF ZOONOTIC PARASITE TRANSMISSION ALONG BUSHMEAT TRADE CHAINS IN NORTHERN GHANA

Michael Akoto-Sasu, Kofi Agyapong Addo, Papa Kofi Amissah-Reynolds

University of Skills Training and Entrepreneurial Development, Mampong-Ashanti, Ghana

This study examined the transmission risk of zoonotic hookworms along bushmeat trade chains in Northern Ghana. A cross-sectional One Health approach was used to combine animal, environmental, and human data. A total of 158 bushmeat samples were collected from four wildlife species: *Thryonomys swinderianus*, *Cricetomys gambianus*, *Tragelaphus scriptus*, and *Civettictis civetta*. Samples were obtained from key trade points, including source communities, markets, transport hubs, and urban centres. Environmental and human exposure data were also collected. Parasitological analysis was done using flotation methods to detect parasite eggs. Seven different parasite species were identified. Hookworm eggs were found in 38 (24.1%) samples. *Toxocara* spp. were found in 29 (18.4%) samples. *Ascaris* spp. were detected in 18 (11.4%) samples. *Strongyloides* spp. were found in 14 (8.9%) samples. *Trichuris* spp. appeared in 12 (7.6%) samples. *Capillaria* spp. were seen in 10 (6.3%) samples, while *Taenia* spp. were detected in 8 (5.1%) samples. Co-infection occurred in 9 (5.7%) samples. Samples that tested positive for hookworm were analysed further. DNA was extracted and amplified using PCR targeting the ITS1–5.8S–ITS2 region. Successful amplification was achieved in 19 (82.6%) samples. Sequencing confirmed zoonotic hookworm species, including *Ancylostoma* spp. and *Uncinaria* spp. Phylogenetic analysis showed that isolates from different hosts clustered together. This suggests possible cross-species transmission along the trade chain. Environmental contamination was also observed. Parasite eggs were found in 12 (9.5%) of soil and surface samples. Risk analysis showed that poor hygiene (OR = 3.2, 95% CI: 1.4–7.1, $p < 0.05$), presence of flies (OR = 2.7, 95% CI: 1.2–6.0, $p < 0.05$), and unsafe meat handling (OR = 4.1, 95% CI: 1.8–9.3, $p < 0.01$) were significant predictors of infection. This study shows that bushmeat trade systems carry significant zoonotic risks. It indicates the need for better hygiene practices and stronger surveillance using a One Health approach.

6403

SPECIES-SPECIFIC TRANSCRIPTIONAL RESPONSES TO HELMINTH INFECTION IN BAAKA HUNTER-GATHERERS AND SYMPATRIC WESTERN LOWLAND GORILLAS (*GORILLA GORILLA GORILLA*), SOUTHEASTERN CAMEROON

Miarisoa L. Ramilison[1], Francis C. Garrett[1], Amber Eliza Trujillo[2], Christina Bergey[1]

[1]Rutgers University, Piscataway, NJ, United States, [2]New York University, New York, NY, United States

Soil-transmitted helminth infections affect millions of people globally, with highest prevalence in tropical regions. Understanding host-parasite interactions across primate species can provide immune mechanisms relevant to control strategies. BaAka rainforest hunter-gatherers and western lowland gorillas (*Gorilla gorilla gorilla*) living sympatrically in southeastern Cameroon face similar helminth exposure due to overlapping rainforest habitat. This affords us a unique opportunity to examine species-specific immune responses. We used dual transcriptomic methods to assay host and parasite gene expression from fecal RNA-seq data. We analyzed samples from BaAka individuals (n=6) and gorillas (n=6) from sympatric populations. After quality filtration, we taxonomically classified all reads using Kraken2. The count of reads classified as helminths served as a proxy for parasite load, allowing differential gene expression analysis. Gorillas showed higher *Strongyloides stercoralis* abundance while humans had elevated *Ascaris lumbricoides*. Genes responsive to *Trichuris trichiura* in humans were enriched for arachidonic acid binding (adjusted $p=5.83\times10^{-3}$) and inflammatory response pathways (adj. $p=6.96\times10^{-3}$). We compared proportions of selected genes between worm response genes and background sets of genes by testing whether helminth response genes were more likely than expected by chance to be under selection in the human population of rainforest hunter-gatherers. Genes with expression responsive to *Ancylostoma duodenale* and *A. lumbricoides* were over-represented among genes under selection in this population (binomial test, p=0.006, p=0.002). Our findings reveal species-specific transcriptional responses to helminth infection and suggest that helminth response genes have been targets of positive selection in hunter-gatherer populations.

6404

HYBRID SCHISTOSOMES ASSOCIATION WITH REDUCED PRAZIQUANTEL EFFICACY DURING S*CHISTOSOMA HAEMATOBIUM* INFECTION IN MALI

Privat Agniwo[1], Safiatou Doumbo Niaré[1], Laurent Dembele[2]

[1]Malaria Research and Training Center (MRTC), Université des Sciences, des Techniques et des Technolo, Bamako, Mali, [2]Malaria Research and Training Center (MRTC), Faculté de Pharmacie, Université de, Bamako, Mali

Urogenital schistosomiasis remains a major public health problem in Mali despite several decades of praziquantel (PZQ) based mass drug administration. Genetic factors involving schistosomes hybridization may contribute to the parasite reduced susceptibility to PZQ. This study aimed to assess the role of *Schistosoma haematobium* group hybrids in the reduced efficacy of PZQ among children aged 6-14 years in Mali. The current study targeted two schistosomiasis endemic villages in Kayes region (Diakalèl and Fangouné Bamamnan) in the western part of the Mali. A total of 364 children were screened using urine filtration. Among

them, 104 positive children were enrolled in the study and followed for up to seven days (Day 7) and and twenty days (Day 20) after treatment with praziquantel (40 mg/kg). Sociodemographic, socioeconomic, and clinical data were collected from these children. Miracidia were collected on FTA cards at Day 0 (pre-treatment) and Day 20 (post-treatment). Parasite profiles were determined following Chelex DNA extraction and PCR amplification of mitochondrial (cox1) and nuclear (ITS and 18S) markers using Rapid Diagnostic PCR (RD-PCR) and Amplification Refractory Mutation System PCR (ARMS-PCR), respectively. The overall prevalence of urogenital schistosomiasis was 53.3%, that was found higher specifically in Diakalèl (85.6%). Cure rates were low, reaching 19.5% at Day 7 and 61.7% at Day 20, remaining below the WHO efficacy threshold (≥90%). However, the study revealed high egg reduction rates (>85%) consistent with the WHO threshold. Rainwater was the main domestic water source, and bathing was the most frequent water-contact activity. Most children presented with terminal hematuria (66.35%), dysuria (94.23%), pollakiuria (50.96%), and abdominal pain (62.5%). The proportion of *S. haematobium* × *S. bovis* hybrids increased markedly from 45.2% before treatment to 72.2% after PZQ administration in the study enrolled population. These findings indicate reduced field efficacy of praziquantel and suggest that hybrid schistosomes display lower suceptibility to PZQ in Mali.

6405

DISCOVERY OF A NEW TRIPLE HYBRID BETWEEN *SCHISTOSOMA MANSONI*, *S. HAEMATOBIUM*AND *S. BOVIS:* WHAT ARE THE IMPLICATIONS FOR SCHISTOSOMIASIS CONTROL AND ELIMINATION?

William K. Anyan[1], Ninette Owusu-Adzorah[2], Boris A. E. Sègnito A. E. S. Savassi[3], Ivy E. L. Bentil[1], Frank Baiden[2], Jerome Boissier[3], Naa Adjeley Frempong[1], David Courtin[4]

[1]Noguchi Memorial Institute for Medical Research, Accra, Ghana, [2]University Health and Allied Sciences, Ho, Ghana, [3]University of Perpignan, Perpignan, France, [4]Insitute of Research and Development, Marseilli, France

Schistosomiasis, a neglected tropical disease is a major challenge for both public and veterinary health. The ability of schistosomes to hybridise has been known since the 1990s. In the last twenty year, with the rise of molecular biology techniques, there have been several publications on hybrid schistosomes from West, Central and East Africa as well as in Europe. Hybrids has mainly been between *Schistosoma haematobium* and *S. bovis*, and in a few instance between the two major species infecting humans in Africa- *S. mansoni* and *S. haematobium*. Indeed schistosome hybridisation is becoming a genuine public health concern, with fears of an emerging zoonotic transmission, and potential increase in virulence traits. Using recently developed molecular methods that enable the screening of a large number of parasites, we have identified a new and particularly worrying triple hybrid between *S. haematobium*, *S. mansoni* and *S. bovis*, in the urine of two young Ghanaian patients. Our finding could have significant epidemiological implications for parasite control and elimination if the intermediate host for these parasites become susceptible to the triple hybrid and it is also able to infect humans and animals.This finding is should be of exceptional interest to the scientific community as it could help guide WHO decisions regarding the revision of its schistosomiasis elimination program. Also it may encourage the adoption of a “One Health” approach in schistosomiasis research, thereby ensuring reliable data collection and enabling optimal disease control.

6406

TRICLABENDAZOLE TREATMENT FAILURE IN PERSISTENT *FASCIOLA HEPATICA* INFECTION: A PEDIATRIC CASE FROM THE COMMUNITY OF POLLOC, CAJAMARCA, PERU

Pedro Ortiz[1], César A. Murga-Moreno[1], David Ruiz-Pérez[1], Cristian Hoban[1], Dayana M. Terrones-Cerna[1], Ana M. Fernández-Sánchez[1], Jhover Diaz-Muñoz[1], Sandra Quispe-Chuquiruna[1], Fabiano Cruzado-Chávez[1], José Aliaga-Tambo[1], Angie Chávez-Pérez[1], Alejandra Hoyos-Sangay[1], Miguel M. Cabada[2]

[1]Centro de Investigación en Medicina Tropical: Fascioliasis, Facultad de Ciencias Veterinarias, Universidad Nacional de Cajamarca, Cajamarca, Peru, [2]University of Texas Medical Branch, Galveston, TX, United States

A 13-year-old girl from the rural community of Polloc, Cajamarca, Peru, an area highly endemic for Fasciola hepatica infection among schoolchildren, was diagnosed in July 2024 via Kato-Katz stool exam showing 100 eggs per gram (epg). She remained egg-positive for 2 years despite 5 triclabendazole (TCBZ) courses. A two-year parasitological follow-up was conducted at six-month intervals. Faecal egg counts decreased from 100 epg at baseline to 80 epg and 40 epg at the second and third evaluations, respectively, but rose again to 60 epg at the fourth assessment. Intermittent abdominal cramping and headache remained the predominant clinical manifestations throughout the follow-up period. Blood tests, liver enzyme levels (including ALP, AST, and ALT) remaining within reference ranges. However, an increase in the absolute eosinophil count was observed, rising from 0.14 to 0.47 × 10^3/µL and remained stable at the third evaluation. In contrast, haemoglobin levels showed a progressive decline from 16.7 to 14.7 g/dL. Anthropometric measurements were within normal limits. Over the same period, height increased from 138 to 143 cm, and weight from 45 to 50 kg. Treatment with TCBZ was administered as follows: i) rounds 1-2: 10 mg/kg/day for 2 days (postprandial); ii) rounds 3-4: 10 mg/kg every 12 hours for 2 days; and iii) round 5: 10 mg/kg/day every 24 hours for 5 days. During the fifth round, the patient required hospital admission due to clinical deterioration, presenting with nausea, colic, vomiting, and diarrhea. On the second day of the final treatment round, the patient expelled 25 adult parasites in her stool, followed by an additional nine parasites on the third day (photographic documentation available). Nevertheless, one month after completion of treatment, eggs remained detectable (60 epg), confirming treatment failure with TCB.

6407

EVALUATION OF A URINE-BASED QPCR ASSAY FOR THE DETECTION OF *FASCIOLA HEPATICA* CELL-FREE DNA (CFDNA) IN SAMPLES FROM CUSCO, PERU

Diego M Ramos-Lette[1], Martha V Fernández-Baca[1], Maria Luisa Morales[2], Melinda B. Tanabe[3], Pedro Ortiz[4], A. Clinton White Jr[3], **Alejandro Castellanos-Gonzalez**[3], Miguel Mauricio Cabada[3]

[1]Universidad Peruana Cayetano Heredia-University of Texas Medical Branch Collaborative Research Center-Cusco, Cusco, Peru, [2]Universidad Peruana Cayetano Heredia, Cusco, Peru, [3]University of Texas Medical Branch, Galveston, TX, United States, [4]Universidad Nacional de Cajamarca, Cajamarca, Peru

Human fascioliasis, a zoonotic disease of global importance, remains endemic in Peru with a high prevalence in rural regions. Current diagnostics rely on labor-intensive microscopy (Kato-Katz) or invasive serology, which often fail to distinguish active from past infections and face low community acceptance. This study evaluates a non-invasive qPCR assay to detect *Fasciola hepatica* cell-free DNA (cfDNA) in urine as a patient-friendly alternative for rural settings. A qPCR assay targeting the ITS region was implemented, with initial standardization using urine samples spiked with 1.6 µg of parasite genomic DNA. For clinical evaluation, 70 urine samples from Cusco and Cajamarca, Peru, were processed using Zymo Research Quick-Urine DNA buffers (70 µL buffer per 1 mL urine), and DNA concentration was quantified via NanoDrop spectrophotometry. Samples were analyzed via qPCR in triplicate and compared against a microscopy “gold standard” requiring three negative Kato-Katz and sedimentation results for a negative

classification. Our results demonstrated that qPCR identified 23 true positives and 26 true negatives, yielding a sensitivity of 85.19% (95% CI: 71.79%-98.59%) and a specificity of 60.47% (95% CI: 45.85%-75.08%). Statistical analysis showed moderate agreement between the two methods (kappa = 0.41, 95% CI: 0.211-0.627). The identification of 17 qPCR-positive/microscopy-negative samples suggests that urine-based molecular detection may uncover sub-microscopic infections missed by traditional parasitology. In conclusion, while microscopy remains the conventional standard, this urine-based qPCR approach offers a sensitive, non-invasive alternative that could significantly improve early diagnosis and facilitate large-scale "One Health" surveillance in resource-limited endemic regions.

6408

ORAL ISOXAZOLINES GIVEN TO DOGS INDUCE HIGH MORTALITY IN *TRIATOMA GERSTECKERI*, VECTORS OF *TRYPANOSOMA CRUZI*

Rachel E. Busselman, Keswick C. Killets, Ashley B. Saunders, Gabriel Hamer, Sarah Hamer

Texas A&M University, College Station, TX, United States

Chagas disease is caused by the parasite *Trypanosoma cruzi*, spread by triatomine ('kissing bug') insects, and impacts over 6 million humans across the Americas. While traditional insecticide residual spraying can reduce human disease in settings where triatomines frequently colonize homes, vector control in areas with largely outdoor/sylvatic triatomines or with pyrethroid-resistant triatomine populations can be challenging. Host-targeted insecticides focused on companion animals may be an effective method for controlling triatomine populations and disrupting *T. cruzi* transmission. We have previously shown triatomines experience rapid mortality when fed blood from dogs treated with fluralaner or lotilaner. We tested the efficacy of two 30-day isoxazoline flea and tick preventions - afoxolaner and sarolaner - on *Triatoma gerstaeckeri*, a predominant vector in the southern US, through and beyond their prescribed efficacy period. Client-owned dogs were given afoxolaner or sarolaner either once or every 30 days for 3 months, and blood samples were collected on day 0 (control), 7, 30, and 90. *Triatoma gerstaeckeri* nymphs were fed on treated blood and monitored daily for 7 days (to assess acute effects) and weekly until death (to assess long-term effects). Among 400 *T. gerstaeckeri* used in trials, 79% of nymphs fed. When feeding on either sarolaner or afoxolaner, triatomine mortality ranged from 97-100% at 7 days, 87-94% at 30 days, to only 50%-63% at 90 days post-treatment. This highlights the acute mortality effects of both preventions during their efficacy period and their waning effects over time. Among triatomines that survived, those fed on treated blood (n=27) had shorter long-term survival (mean = 144 days; range = 15-423 days) than control insects (n=87) (mean 234 days; range = 16-270 days) (p-value<0.001). Overall, laboratory trials of isoxazolines continue to show great efficacy in killing triatomine vectors; randomized controlled field trials are needed to show their impact on host infections under natural conditions.

6409

GENETIC VARIABILITY WITHIN *TRIATOMA SANGUISUGA* COMPLEX IN NORTH AMERICA AND THE RE-DESCRIPTION OF TRIATOMA AMBIGUA (NEIVA 1911) IN FLORIDA, USA

Norman L. Beatty[1], Sebastiàn Pita[2], Bruno Agustín Sansoni Ruidíaz[2], Sergio Méndez-Cardona[3], Bernardo Moreno Peniche[4], Chanakya Ravi Bhosale[5], Susan Halbert[6], Catherine Nance[6], Paul Skelly[6], Francisco Panzera[2], Eva Nováková[7], Samantha Wisely[8], Rachel Curtis-Robles[9], Md Mainul Hasan Sarker[10], Juan David Ramirez Gonzalez[10], Gabriel Hamer[11], Sarah Hamer[9]

[1]University of Florida, Division of Infectious Diseases and Global Medicine, Gainesville, FL, United States, [2]Sección Genética Evolutiva, Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay, [3]Florida Medical Entomology Laboratory, University of Florida, Vero Beach, FL, United States, [4]University of California, Berkeley, CA, United States, [5]Nova Southeastern University, Fort Lauderdale, FL, United States, [6]Florida Department of Agricultural and Consumer Services, Gainesville, FL, United States, [7]Faculty of Science, University of South Bohemia, Ceske Budejovice, Czech Republic, [8]Department of Wildlife Ecology and Conservation, University of Florida, Gainesville, FL, United States, [9]Department of Veterinary Integrative Biomedical Sciences, Texas A&M University, College Station, TX, United States, [10]College of Public Health, University of South Florida, Tampa, FL, United States, [11]Department of Entomology, Texas A&M University, College Station, TX, United States

Our team investigated genetic and morphological diversity within the *Triatoma sanguisuga* complex across the southeastern United States to clarify taxonomic structure and implications for Chagas disease risk. Using mitochondrial (cytb) and nuclear (ITS-2) markers from 173 available specimens, we identified 113 haplotypes with high haplotype diversity (Hd=0.980) and four well-supported genetic lineages. A distinct Florida-restricted lineage demonstrated substantial divergence from other groups (K2P >7.5%), supported consistently across multiple species delimitation methods. Phylogenetic analyses confirmed strong clustering of this lineage, suggesting evolutionary separation. Morphometric analyses of 82 adult specimens revealed significant and reproducible differences between Florida and non-Florida populations. Linear discriminant analysis showed clear separation with high classification accuracy, further supported by random forest modeling. Key distinguishing features included reduced synthlipsis width and a consistent ventral connexivum coloration pattern in Florida specimens. Diagnostic thresholds for synthlipsis demonstrated excellent discriminatory performance (AUC up to 0.997), enabling reliable differentiation between taxa. Integrating molecular and morphometric evidence, our findings support revalidation of *T. ambigua* (Neiva, 1911) as a distinct species within the *T. sanguisuga* complex. This taxonomic clarification has important epidemiologic implications, as Florida populations exhibit frequent human-vector contact. Recognition of cryptic species diversity within North American triatomines refines our understanding of vector ecology and transmission dynamics, and highlights the need for region-specific surveillance strategies. These findings underscore that what was previously considered a single widespread vector likely represents multiple biologically distinct taxa with differing public health significance.

6410

EXPERIMENTAL EVIDENCE SUPPORTING SECONDARY VECTORS IN VISCERAL LEISHMANIASIS TRANSMISSION IN BRAZIL

Fredy Galvis Ovallos[1], Amanda Andrade do Rosario[1], Gledson Bandeira Maia[1], Marcia Dalastra Laurenti[2], Roberto Mitsuyoshi Hiramoto[3], Claudio Casanova[4]

[1]School of Public Health, University of São Paulo, São Paulo, Brazil, [2]Medical School, University of São Paulo, São Paulo, Brazil, [3]Adolfo Lutz Institute, São Paulo, Brazil, [4]Pasteur Institute, São Paulo, Brazil

Visceral leishmaniasis (VL) is a neglected vector-borne disease caused by infection with *Le. infantum*, primarily transmitted by the sand fly L*utzomyia longipalpis*, which typically acquires the parasite from domestic dogs. Brazil accounts for approximately 95% of VL cases reported annually in the Americas. In recent decades, a pattern of geographic expansion has been observed, including the emergence of new transmission foci in areas where the primary vector is absent. Therefore, this study aimed to experimentally assess the susceptibility of potential secondary vectors to *Le. infantum* infection and to evaluate their implications for the spread of VL in Brazil. Ten species from six genera were fed through artificial chicken skin membranes containing *Le. infantum* promastigotes (10^6), and parasite development and survival after blood digestion were evaluated. Seven species, including L*u. longipalpis*, were susceptible to infection and supported parasite development. Infection rates ranged from 1.8% to 66.7%, with *Pintomyia fischeri* (66.7) and *Nyssomyia whitmani* (40.4) showing the highest rates. Intermediate infection rates were observed in *Pintomyia pessoai* (17.6%), *Nyssomyia neivai* (16.1%), and *Migonemyia migonei* (10.5%). However, infective metacyclic forms were observed only in *Pi. fischeri*, *Ny. neivai* and *Pi. pessoai*, indicating their potential vector competence. Considering that canine and human cases had been reported in areas where those species occur, our findings bring evidence that the transmission of VL could be

sustained by this secondary vector species. Introduction of infected dogs in those areas with putative vectors could be a primary factor in the spreading of VL with important epidemiological implications.

6411

DEER ARE NOT THE MOST IMPORTANT BLOODMEAL HOST FOR LARVAL LONE STAR TICKS FROM MASSACHUSETTS

Heidi K. Goethert[1], Patrick Roden-Reynolds[2], Sam R. Telford[3]

[1]Tufts Univ Sch of Veterinary Med, North Grafton, MA, United States, [2]Martha's Vineyard Tick-borne Illness Reduction Initiative, West Tisbury, MA, United States, [3]Tufts University, Department of Infectious Disease & Global Health, North Grafton, MA, United States

The Lone Star tick (LST), *Amblyomma americanum*, commonly bites humans across much of the eastern U.S., with some newly established infestations in Massachusetts. LSTs serve as vectors for diverse infections, including those caused by *Ehrlichia chaffeensis* and Heartland virus. Their bite is also associated with alpha-gal syndrome, an acquired allergy to red meat. This disease is rapidly becoming as important as Lyme disease in areas where both diseases occur. All three stages of LST are thought to feed primarily on deer in most sites, but host contributions have not been quantitatively analyzed, particularly where they are newly invasive. To this end, we collected host-seeking LST nymphs and adults from two sites in Massachusetts, one with a small deer herd (GI), and one with many deer (MV); both have well established infestations of deer ticks. We determined the identity of hosts upon which the ticks had fed on during their previous instar using our published retrotransposon-based bloodmeal remnant identification qPCR assay. We tested 38 nymphs and 39 adults from GI and 52 nymphs and 44 adults from MV for evidence of having fed on mouse, vole, rabbit, bird, deer, turkey, shrew, skunk/raccoon, opossum, squirrel/chipmunk, and cat. On GI, 39% of the nymphal ticks had fed as larvae on squirrel/chipmunk and 21% on rabbits, whereas only 10.5% had fed on deer. Interestingly, 33% of the adult ticks had fed as nymphs on cats and 30.8% had fed on deer. On MV, 38% of nymphs had fed as larvae on rabbits and 31% on turkeys, but only 11.5% had fed on deer. The adult LST had fed as nymphs primarily on deer (36.4%), while remnant DNA of cat and turkey were both detected in 18.2%. We conclude that although deer commonly fed LST nymphs, they were unexpectedly not highly utilized by the larvae at either field site. It is clear that hosts other than deer, such as rabbits, squirrels, turkeys and cats are able to help sustain LST populations. As with deer ticks, host utilization is site-specific and highly dependent on the local vertebrate community.

6412

TEXAS STATEWIDE TICK SURVEILLANCE THROUGH COMMUNITY ENGAGEMENT AND CITIZEN SCIENCE

Megan M. Duffey[1], Fernique Pinder[1], Maya Murry[1], Alisa Nelson[1], Morgan Jibowu[2], Paige Lillibridge[1], Alexander Kneubehl[1], Sarah M. Gunter[1]

[1]Baylor College of Medicine, Houston, TX, United States, [2]Houston, TX, United States

Tick-borne diseases are both a persistent and growing public health concern worldwide. In recent years, the geographic range of established tick species has expanded, and invasive tick species have emerged, reshaping the epidemiology of tick-borne disease. To better understand the epidemiology and risks of tick-borne diseases in Texas, we have established a state-wide citizen science-based tick surveillance and pathogen-testing program. This initiative integrates community-based collection with advanced molecular epidemiology techniques to characterize tick distribution and pathogen prevalence across diverse ecological regions of the state. Additionally, this study functions as a community resource, offering free tick identification and pathogen testing for individuals and empowers residents to contribute valuable data. We solicited ticks from the public using a website and targeted social media advertisements. We also collaborated with 18 of the Texas Wildlife Management Areas run by Texas Parks and Wildlife to obtain ticks collected from hunter-harvested animals. Ticks were identified to species level by microscopy, bisected, and underwent DNA extraction. Extracted DNA was then tested by qPCR for tickborne pathogens including *Rickettsia* spp., *Ehrlichia* spp., and *Borrelia* spp. A total of 1,068 ticks have been submitted for analysis from November 2023 to February 2025. Ten unique tick species have been submitted from 254 Texas counties. Pathogen testing has identified 34% (363/1068) of ticks as *Rickettsia* positive, 7% (75/1068) as *Ehrlichia* positive, and no samples have tested positive for *Borrelia*. By leveraging community engagement and modern molecular tools, this citizen-driven surveillance model strengthens early detection capacity, enhances situational awareness, and supports more targeted public health responses to mitigate the growing impact of tick-borne diseases in Texas.

6413

TARGETING EMBRYO-SPECIFIC GENES IN *HAEMAPHYSALIS LONGICORNIS* FOR NEXT-GENERATION VECTOR CONTROL

Kofi Dadzie Kwofie[1], Hayato Kawada[1], Yuki Koike[2], Sana Sasaki[2], Kei Jimbo[2], Keiko Mizutani[2], Fusako Mikami[1], Naotoshi Tsuji[1], Takeshi Hatta[1]

[1]Department of Parasitology and Tropical Medicine, Kitasato University School of Medicine, Sagamihara, Japan, [2]Department of Molecular and Cellular Parasitology., Graduate School of Medicine, Kitasato University School of Medicine, Sagamihara, Japan

Tick-borne diseases (TBDs), including Severe Fever with Thrombocytopenia Syndrome (SFTS), pose an increasing threat to public health and agriculture in Japan, with fatality rates reaching up to 30%. Climate change and ecological shifts are expanding tick habitats, while current control strategies, such as acaricides and personal protection, are becoming less effective due to resistance and environmental constraints. These challenges emphasize the urgent need for innovative and targeted tick control approaches. This study investigates embryogenesis, a critical, yet underexplored stage in the life cycle of *Haemaphysalis longicornis*, as a novel point of intervention. Embryogenesis spans approximately 20-25 days under optimal conditions and was classified into early (0-5 days post-oviposition [dpo]), mid (11-15 dpo), and late (21-25 dpo) stages. A custom microarray was developed using expressed sequence tags derived from cDNA libraries of salivary glands, midgut, ovary, and embryos. RNA extracted from eggs at early, mid-, and late embryonic stages was analyzed, with a focus on identifying genes upregulated specifically during the mid-stage, to avoid genes with overlapping maternal or larval functions. Clustering analysis identified 31 genes upregulated during mid-embryogenesis. Validation by RT-PCR and RT-qPCR confirmed strong embryonic expression, with 11 uncharacterized genes peaking at 13 dpo. Functional analysis of one candidate gene, S03044-17L21, using parental RNA interference significantly reduced transcript levels and caused developmental defects in approximately 60% of eggs, resulting in hatching failure. These findings highlight embryo-specific genes as promising molecular targets for next-generation, species-specific, and environmentally sustainable tick control strategies, including anti-tick vaccines and spray-induced gene silencing technologies.

6414

DIFFERENTIAL PRODUCTION OF TICK SALIVA MICRORNAS ASSOCIATED WITH *RICKETTSIA* INFECTION

Shovon Lal Sarkar, Kevin Macaluso

University of South Alabama, Mobile, AL, United States

The Gulf Coast tick, *Amblyomma maculatum*, is an aggressive tick that transmits *Rickettsia parkeri*, a human pathogen that causes spotted fever group rickettsiosis. Transmission of rickettsial agents occurs via tick saliva, which also contains non-proteinaceous molecules, including miRNAs. In ticks, miRNAs regulate biological, molecular, and cellular processes and vary with pathogens. Manipulation of miRNAs can influence tick development, feeding activity, and pathogen transmission across different species. While previous studies have examined the importance of miRNAs in ticks, relatively little is known about the presence of miRNAs in saliva.

We hypothesize that rickettsial infection in the tick will induce a distinct miRNA profile. To test this hypothesis, a comprehensive profile of saliva miRNAs from *A. maculatum* infected with *R. parkeri* was compared to salivary miRNAs from uninfected ticks. Saliva from 54 ticks was used to isolate, quantify, and sequence miRNAs with Illumina single-end 50 bp sRNA sequencing. Raw data showed robust miRNA profiles (>18 nt) with 72675253 reads in uninfected and 61953996 reads in *R. parkeri*-infected tick saliva. Preliminary data indicate that, out of 110 highly confident miRNAs, 7 miRNAs were upregulated, and 9 miRNAs were downregulated in *Rickettsia*-infected saliva, compared to uninfected tick. Among significantly differentially expressed miRNAs, Ama-miR-275, Ama-let-7, and Ama-miR-1 were selected for *in silico* functional characterization. Gene Ontology and network analyses of differentially expressed miRNAs' target host genes predicted roles in the regulation of metabolic processes, protein binding, development, cellular component biogenesis, and subcellular localization. Ongoing studies will employ *in vivo* RNAi assays using miRNA mimics (AgomiR) or anti-miRNAs (AntagomiR) targeting specific miRNAs to elucidate the functions of saliva-specific miRNAs in tick feeding and pathogen transmission. Ultimately, the goal is to functionally characterize differentially expressed miRNAs to identify intervention strategies for tick-borne rickettsial diseases.

6415

SPOTTED FEVER GROUP *RICKETTSIA* TRANSMISSION/ DISSEMINATION KINETICS AND CUTANEOUS HOST RESPONSE DURING SINGLE TICK FEEDING EVENTS

Chanakan Suwanbongkot[1], Ingeborg M. Langohr[2], Killian Z. Brewer[1], Barrington A. Robert[1], Kevin Macaluso[1]

[1]University of South Alabama, Mobile, AL, United States, [2]Global Discovery Pathology & Multimodal Imaging, Translation Models Research Platform, Sanofi, MA, United States

Rickettsia parkeri, an emerging tick-borne pathogen, is transmitted by *Amblyomma maculatum.* Initial bacterial inoculation occurs in the skin via infected tick bite. During blood acquisition, tick salivary factors manipulate host hemostatic and immune responses. Examination of rickettsial pathogenesis and the host response to infection in murine models primarily utilize needle inoculation. As a result, biological variables including transmission dynamics and the influence of vector saliva on the host response at the infection site are understudied. To test the hypothesis that a distinct host cutaneous response will be elicited based on the infection status of the tick, a single tick feeding model was developed to assess the transmission and dissemination kinetics of *R. parkeri* from the early stage of tick attachment (1 hour) to fast feeding stage (144 hours). Furthermore, changes in the cutaneous immune response were characterized through immunophenotyping, cytokine and chemokine profiling, and histopathological analysis. The data show transmission of *R. parkeri* from the infected tick to the host occurs within 1 hour post-tick attachment (hpa), with rapid dissemination to internal tissues and lymph nodes. The quantifiable bacterial load in the mouse skin delivered by an infected tick was 9,500 bacteria at 3 hours, increasing to 15,000 and 22,000 rickettsiae at 48 and 144 hours, respectively. Independent of tick infection status, the degree of time-dependent inflammation was similar at 3- and 48 hpa. However, in *R. parkeri*-infected skin at 144 hpa, macrophages were the dominant cell type, with enhanced levels of IFN-γ and TNF-α while IL-10 production was reduced. The data suggest delayed pro-inflammatory activation in response to rickettsial infection when delivered by ticks. This study provides valuable insights into the early events of SFG *Rickettsia* transmission and the host response, assisting in the development of preventive strategies against tick-borne rickettsial disease.

6416

HIGH-THROUGHPUT *SIMULIUM* BLACKFLY CLASSIFICATION USING ARTIFICIAL INTELLIGENCE-ASSISTED MICROSCOPY

Peter K. Ward[1], John Bergelin[1], Henok Birhanu[2], Emily Griswold[3], Jenna E. Coalson[3], Aderajew Mohammed[2], Anley Haile[2], Joseph P. Shott[1], Peter Dahlberg[1], Zerihun Tadesse[2], Gregory S. Noland[3], Karen E.S. Hamre[3]

[1]Enaiblers, Uppsala, Sweden, [2]The Carter Center - Ethiopia, Addis Ababa, Ethiopia, [3]The Carter Center, Atlanta, GA, United States

Onchocerciasis (river blindness) is a neglected tropical disease (NTD) transmitted to humans through the bites of infected *Simulium* blackflies. The World Health Organization strategy for onchocerciasis elimination depends on entomological surveillance of *Simulium* vectors. Vector collections and laboratory processing require sorting and pooling of flies by species; time-consuming, labor-intensive tasks prone to fatigue-related error. AI-assisted microscopy may reduce the potential for species misclassification and subsequent inclusion of non-vectors in test pools. A diagnostic prototype to scan stool, urine, and blood slides to detect other NTDs using an AI-powered digital pathology platform with automatic slide reading and human-in-the-loop verification was adapted to evaluate the feasibility of integrating blackfly classification into the AI microscope. Custom cartridges and new boundary detection algorithms were developed. In May 2025, 890 alcohol-preserved blackflies collected through human landing catches for surveillance by the Ethiopia Onchocerciasis Elimination Program were scanned and images annotated by entomologists at the Ethiopian Public Health Institute. Results were compared with classifications from conventional microscopy. Cross-validation showed strong species-level classification performance: precision and recall of 93.4% and 92.5% for *S. bovis* (n=287), 86.8% and 78.3% for *S. ethiopiensis* (n=167), 91.2% and 83.6% for *S. damnosum* (n=286), and 79.1% and 88.2% for *S. dentulosum* (n=150). A correlation between the quantity of training data and model performance was observed. Scanning, image flattening, stitching, and analysis yielded a throughput of ~20 flies per hour per scanner - 1 microscopist operating 4 scanners yields ~80 flies/hour. Opportunities exist for technical, operational, and performance improvements. When optimized, the AI microscope may reduce misclassification during molecular xenomonitoring, improve accuracy of program monitoring, and strengthen targeting of resources. More broadly, it demonstrates the potential for an integrated epi-ento NTD surveillance tool.

6417

MAPPING ZOONOTIC SPILLOVER RISK IN SUB-SAHARAN AFRICA: A SYSTEMATIC REVIEW OF BAT AND RODENT VIRUS SURVEILLANCE AT HUMAN WILDLIFE INTERFACES

Frank Lartey[1], Philip Sanjok[2], Araba Aso Odum[3]

[1]UC Berkeley, Berkeley, CA, United States, [2]Euracare Hospital Ghana, Tarkwa, Ghana, [3]Nyaho Hospital, Accra, Ghana

Sub-Saharan Africa accounts for the majority of documented zoonotic viral spillover events globally, yet existing systematic reviews focus predominantly on post-outbreak response rather than proactive risk characterization. No published review has integrated wildlife virome surveillance, human serosurvey evidence, and community behavioral risk data into a unified spillover risk framework for West and Central Africa. This systematic review addresses that gap by synthesizing published metagenomics, serological, and behavioral surveillance studies from the region. A structured literature search yielded virome studies encompassing 846 wild small mammals from Sierra Leone and 1,094 bat and rodent specimens from East Africa, documenting 39 RNA viruses, including 26 novel taxa, with Paramyxoviridae showing the highest species diversity across all host orders. Epidemiological synthesis across 52 outbreak reports from 25 Sub-Saharan African countries documented a pooled human attack rate of 54.4 per 1,000 persons, with Marburg virus, Ebola virus, and Lassa fever virus recording the highest case fatality rates. Serosurvey data from West Africa confirm measurable human exposure to reservoir-borne pathogens in communities

with documented wildlife contact, while behavioral analyses identify bushmeat handling, proximity to bat roosting sites, and land use change as the primary spillover exposure drivers. Integrated analysis of these three data streams reveals a convergent risk profile centered on bat and rodent reservoir communities at forest and agricultural boundaries. These findings establish an evidence-based framework for targeting proactive surveillance and community-level interventions at the highest priority spillover zones across the region, filling a critical gap in the existing literature.

6418

SPILLOVER DYNAMICS OF SARS-COV-2 AT THE HUMAN-WILDLIFE INTERFACE: GENOMIC EVIDENCE FROM WHITE-TAILED DEER IN THE WASHINGTON DC METROPOLITAN AREA

Gideon G. Ameh

George Mason University, Fairfax, VA, United States

Cross-species transmission at human-wildlife interfaces is a key driver of emerging infectious disease risk. SARS-CoV-2 has infected multiple mammalian hosts, including white-tailed deer (*Odocoileus virginianus*), raising concern that wildlife could serve as secondary reservoirs with implications for viral persistence, evolution, and spillback to humans. We investigated transmission dynamics in free-ranging deer in the Washington DC Metropolitan Area which includes adjacent counties in Northern Virginia and Maryland representing a densely populated region with frequent human-deer interactions. Whole-genome sequences from deer sampled in Maryland and Virginia in 2024 were analyzed alongside publicly available human and deer genomes (2021-2023). From ~64,000 genomes compiled from GISAID and NCBI, 367 high-quality genomes were retained for time-resolved phylogenetic analysis. We used these data to infer introduction events and assess whether infections in deer reflected repeated spillover from humans or sustained deer-to-deer transmission. Deer-derived genomes were interspersed within human viral diversity rather than forming persistent deer-only clades, supporting repeated human-to-deer spillover as the dominant transmission pattern. Evidence for sustained deer-to-deer transmission was limited. Selection analyses using a branch-site model (codeml) identified signals of positive selection in spike and ORF1a along deer-associated lineages, though these were weak and likely influenced by small sample sizes. Overall, findings indicate that deer in the DMV currently function primarily as spillover hosts. Continued integrated surveillance is essential to detect shifts in transmission dynamics and evolutionary trajectories that could elevate spillback risk.

6419

FIRST REPORT OF CHIKUNGUNYA, YELLOW FEVER AND ZIKA INFECTION IN WILD OLIVE BABOONS AT A HUMAN-WILDLIFE INTERFACE IN GHANA

Patrick Kwasi Obuam[1], Sandra Abankwa Kwarteng[1], Anthony Afum-Adjei Awuah[1], Seth Offei Addo[2], Ernest Ampon[1], Sylvester Coleman[3], Meyir Yiryele Ziekah[4], Samuel Kweku Dadzie[1], Ellis Owusu-Dabo[5]

[1]Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, [2]Noguchi Memorial Institute of Medical Research, University of Ghana, Accra, Ghana, [3]Liverpool, United Kingdom, [4]Forestry Commission, Wildlife Division, Kumasi, Ghana, [5]Kwame Nkrumah University of Sci & Tech, Kumasi, Ghana

Human-wildlife interfaces in protected areas present significant risks for arboviral spillover, particularly for communities living on the periphery of protected parks that also serve as ecotourism sites. In Mole National Park, Ghana, olive baboons (*Papio anubis*) frequently forage in peridomestic spaces, creating opportunities for cross-species transmission, yet integrated surveillance across hosts remains critically limited. To evaluate this risk, we conducted a two-phase eco-epidemiological investigation alongside mosquito surveillance in Murugu, a community on the periphery of the game park, in December 2023 and April 2024. *Aedes* mosquito species were sampled with baited Sentinel traps and mechanical aspirators from the study area and screened for the presence of viral RNA. Sera from Murugu residents, as well as five olive baboons (one each from the 5 different troops) that frequently visit the study community, were collected and screened for detection of IgG antibodies against chikungunya (CHIKV), dengue (DENV), yellow fever (YFV), and Zika virus (ZIKV) using the multiplex enzyme-linked immunosorbent assay (ELISA). None of the *Aedes* mosquitoes (n=663 predominantly *Ae. aegypti*) screened across both seasons tested positive for viral RNA. However, human seroprevalence for CHIKV, DENV, and ZIKV was determined to be 34.1%, 26.4%, and 17.6%, respectively. We anticipated a high seroprevalence for YFV (36.3%) due to the high vaccination in the area. Crucially, active engagement in sylvatic transmission cycles was confirmed in the baboon screened, revealing a 40% CHIKV seroprevalence and a 20% seroprevalence for both YFV and ZIKV. To our knowledge, this is the first report that confirms CHIKV, YFV and ZIKV infections in olive baboons (*Papio anubis*) and as important reservoirs possibly mediating the dissemination of these arboviruses in Ghana. Our findings underscore the importance of continued surveillance and an integrated One Health approach to the control of arboviruses in interventions.

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OUTBREAK OF H9N2 AVIAN INFLUENZA VIRUSES IN LESSER RHEA IN THE ANDEAN HIGHLANDS OF PERU, JUNE-JULY 2025

Mariana Leguia[1], Alejandra Garcia-Glaessner[1], Alvin Crespo-Bellido[2], Breno Muñoz-Saavedra[1], Diana Juarez[1], Patricia Barrera[1], Gabriela Salmon-Mulanovich[1], Shadam Checahuari[3], Dany Cruz[3], Dennis Huisa[4], Grover Idme[4], Martha I. Nelson[2], Jesus Lescano[5]

[1]Pontificia Universidad Católica del Perú, Lima, Peru, [2]National Institutes of Health, Bethesda, MD, United States, [3]Proyecto Especial Binacional Lago Titicaca, Puno, Peru, [4]Administración Técnica Forestal y de Fauna Silvestre, Puno, Peru, [5]Servicio Nacional Forestal y de Fauna Silvestre, Lima, Peru

Avian influenza viruses are endemic in the Americas and responsible for outbreaks in both domestic and wild birds that occasionally spill over into other animals, including humans. We report the first known outbreak of avian influenza H9N2 in lesser rhea (*Rhea pennata*) in the region of Puno in Peru. Lesser rhea, also known as Darwin's rhea, is a large flightless bird up to 100 cm tall that has been declared endangered by the Peruvian government. The animals in this study lived in an isolated conservation center located in remote highlands above 3,800 m.a.s.l. Between June and July 2025, a total of 46/92 animals were recorded sick, with symptoms that included greenish diarrhea (100%), dyspnea (76%), nasal discharge (42%), hyporexia (24%) and drowsiness (18%), and many later died. Gross pathology exams revealed septicemia characterized by severe hepatitis, pneumonia, tracheitis, enteritis, and encephalitis. Swab and necropsy samples tested positive for Influenza A by PCR and were later identified as H9N2 through whole genome sequencing. We generated complete H9N2 genomes for two individuals. No additional pathogens were found in their samples. Phylogenetic analysis across all eight segments revealed that the viruses were low pathogenicity H9N2 influenza strains from North America, which indicated this outbreak represented a new introduction of the virus into South America. We also performed a comparative mutational analysis and identified multiple mutations previously associated with mammalian host adaptation, increased virulence, increased pathogenicity, and increased virus binding to α2-6 receptors, which may explain the high mortality rates observed despite the supposedly low pathogenicity of the strain. This is the first report of a natural H9N2 systemic infection in an avian host, highlighting a need for increased surveillance efforts for zoonotic influenza viruses with pandemic potential.

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NOVEL MULTI-SPECIES SUSCEPTIBLE-INFECTIOUS-RECOVERED (SIR) MODEL OF ZOONOTIC INFLUENZA TRANSMISSION BETWEEN CONCENTRATED ANIMAL FEEDING OPERATIONS (CAFOS), WORKERS, AND SURROUNDING COMMUNITIES

Amanda K. Irish, Eric Kontowicz, Jacob J. Oleson
University Of Iowa, Iowa City, IA, United States

Zoonotic influenza at the agricultural-human interface represents a persistent pandemic threat, yet existing models inadequately capture multi-species dynamics linking concentrated animal feeding operations (CAFOs) to surrounding rural communities. We developed a novel coupled multi-species susceptible-infectious-recovered (SIR) model to characterize influenza transmission within and between typical grow-out swine and layer poultry CAFOs, the agricultural workforce, and rural communities in the US Midwest. The stochastic model incorporates species-specific density-dependent transmission (β) and recovery (γ) parameters with bidirectional cross-species transmission coefficients linking animal populations to workers and workers to the community during typical poultry (69-week) and swine (23-week) production cycles. The community was modeled as a closed population. Parameters were derived from published literature and simulated 483 days of CAFO production. Simulations seeded in swine populations demonstrated rapid within-CAFO epidemics (peak ~day 5, resolution by day 50), an early community outbreak followed by sporadic transmission, and successful swine worker-to-community member transmission in 62% of iterations (n = 5,000). Simulations seeded in poultry showed rapid epidemic growth (peak day 1-2, resolution by day 10-15) without community transmission. Simulations seeded in the community produced a small initial outbreak with intermittent low-level spread to swine and poultry populations across the simulation period. These results demonstrate successful within-CAFO and interspecies transmission, with the hog workforce serving as a critical bridging node between animal reservoirs and rural communities — reinforcing previous work highlighting the need for targeted non-pharmaceutical interventions at this interface. This framework will be extended to evaluate blocking measures (e.g. personal protective equipment and scrub-in/scrub-out disinfection protocols) and integrate stakeholder data on implementation barriers to inform zoonotic influenza surveillance and preparedness.

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ECOLOGICAL AND BEHAVIORAL DRIVERS OF HENIPAVIRUS SPILLOVER IN RURAL COMMUNITIES IN BANGLADESH

Ariful Islam[1], Shusmita Dutta Choudhury[2], Spencer Sterling[3], Monjurul Islam[1], AKM Dawlat Khan[2], Md Ziaur Rahman[4], Jade K Forwood[1], Tahmina Shirin[2], Jonathan H Epstein[5]
[1]Gulbali Research Institute, Charles Sturt University, Wagga Wagga, Australia, [2]Institute of Epidemiology, Disease Control & Research (IEDCR), Dhaka, Bangladesh, [3]HJF, Bethesda, MD, United States, [4]icddr,b, Dhaka, Bangladesh, [5]One Health Science, Mt Kisco, New York, NY, United States

Nipah virus (NiV) is an emerging bat-borne zoonosis causing repeated outbreaks in Bangladesh with >70% mortality, with spillover spatially clustered in Bangladesh's western "Nipah Belt," predominantly linked to consumption of bat-contaminated raw date palm sap (RDPS). However, NiV has been detected in Pteropus medius bats throughout the country, and exposure to other henipaviruses remains poorly understood. Between 2022-2023, we conducted a behavioral and ecological survey and serological surveillance across six districts communities: three within the Nipah Belt and three in outside the Belt in eastern Bangladesh, administering structured questionnaires to 2,400 individuals (400 per site) and testing serum samples using multiplex microsphere-based immunoassays for IgG reactivity against henipavirus envelope glycoproteins, including Mojiang-like virus. Nipah Belt households exhibited significantly higher risk behaviors compared to eastern communities, with RDPS consumption higher in the Belt (55.5% vs 16.8%), along with sap harvesting (13.4% vs 1.6%) and date palm tree ownership (28.6% vs 7.2%). NiV Belt communities also showed higher rates of grazing animals under bat roosts (43.5% vs 15.3%) and consuming bat-bitten fruits (43.2% vs 29%) (all $p<0.001$). Generalized Linear Mixed Effect Models identified gender, education, income, RDPS harvesting, and tree ownership as significant consumption determinants. Serological analysis revealed substantial Mojiang-like henipavirus exposure, with age and agricultural occupation significantly associated with higher antibody levels, suggesting occupational and cumulative exposure patterns. Higher RDPS consumption and multiple human-bat interface behaviors in western regions drive spatial discrepancy in NiV outbreaks, while detection of Mojiang-like virus exposure, particularly among agricultural workers, indicates broader henipavirus circulation beyond NiV, highlighting the need for targeted One Health interventions addressing multiple exposure pathways and expanded henipavirus surveillance in Bangladesh.

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SYNANTHROPIC BATS HARBOR MEDICALLY RELEVANT SANDFLY-BORNE PATHOGENS AT HUMAN-ANIMAL INTERFACES IN KENYA

Irene K. Akunda[1], Jennifer Horton[1], Paul Webala[2], Teryn maccabee[1], Ricardo Rivero[1], James Hassel[3], Joseph Kamau[4], Stephanie Seifert[1]
[1]Washington State University, Pullman, WA, United States, [2]Maasai Mara University, Narok, Kenya, [3]Global Health Program, Smithsonian's National Zoo & Conservation Biology Institute, Washington, D.C., DC, United States, [4]Kenya Institute of Primate Research, Nairobi, Kenya

Synanthropic wildlife can facilitate maintenance and transmission of vector-borne pathogens at the human-animal interface. Sandfly-borne phleboviruses (SBPs) include medically relevant arboviruses associated with febrile and neurological illness. Relative to surveillance in humans and domestic animals, the role of wildlife in the ecology of SBPs and other sandfly-borne pathogens is comparatively understudied. Here we report on the detection of sandfly-borne pathogens by metatranscriptomics from tissue and blood samples collected from diverse bat taxa in the Kenyan Rift Valley. Many of the sampled bat species occupy synanthropic roosting environments, including homes, pit latrines, and abandoned mines, which also harbor phlebotomine sandflies, creating opportunities for bat-vector-human interactions. Viral sequencing identified phlebovirus RNA, and ongoing genomic analyses indicate that these viruses are closely related to known human-pathogenic lineages. Comparative viral growth curves using bat-derived cell lines representing diverse bat taxa demonstrate that this phlebovirus can replicate in cells from Old World bat species. In addition, transcripts corresponding *Leishmania* spp. were detected in bat samples, suggesting that synanthropic bats are exposed to multiple sandfly-borne pathogens in shared ecological settings near human settlements. Together, these findings identify a previously unrecognized synanthropic bat-sandfly interface and suggest that bats may participate in the ecology of sandfly-borne pathogens in East Africa. This work highlights the need for expanded surveillance at human-wildlife interfaces to better understand the contribution of these phleboviruses to undiagnosed human disease in the region.

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DEFORESTATION CLEARING THE WAY FOR SPILLOVER EVENTS: A ONE HEALTH-DRIVEN ANALYSIS OF BOLIVIAN HEMORRHAGIC FEVER AND CHAPARE HEMORRHAGIC FEVER OUTBREAKS AND BOLIVIAN LAND USE POLICY

Alexander V. Alvarado, Caitlin J. Cotter
University of Texas Medical Branch, Galveston, TX, United States

Machupo virus (MACV) and Chapare virus (CHAPV) are New World Mammarenaviruses that cause Bolivian Hemorrhagic Fever (BHF) and Chapare Hemorrhagic Fever (CHHF), respectively, with sporadic outbreaks in Bolivia since 1959 (BHF) and 2003 (CHHF). Due to high case fatality rates and classification of these viruses as category A bioterrorism agents,

understanding the factors driving viral spillover has significant public health and biosecurity implications. While contact between infected rodent reservoirs and humans is thought to be the primary route of transmission, the factors driving rodent-human interactions have not been consistently characterized over time. The intersectional nature of rodent health, human health, ethnogeographic factors, and land use policies are hypothesized to have shaped risk factors leading to the 1962-1964 San Joaquin BHF outbreak, and a confluence of similar factors may have been responsible for subsequent BHF and CHHF outbreaks. Rodent habitat preferences are known to include grasslands and forest-fringe environments for the reservoirs of MACV and CHAPV, respectively. Due to increased rodent populations in peridomestic regions, leading to higher likelihood of infected rodent-human interactions, deforestation is hypothesized as a primary driver of MACV and CHAPV spillover events. This abstract presents a geolocated, spatiotemporal analysis of land cover changes between 2001-2024 in and around locations of BHF and CHHF case clusters, coupled with case reports and land use policy analyses. Results indicate a correlation between recent deforestation events and cases of arenaviral disease. Our analysis of relevant policy and ethnographic changes between 2001-2024 revealed that land colonization practices led to the dispossession of lands by indigenous peoples in Beni, La Paz, and Cochabamba departments, driving land clearance for agriculture, which was associated spatially and temporally with BHF and CHHF outbreaks. Implications of policy changes, such as the 1953 Agrarian Reform Decree, are discussed and compared with resulting land use change and disease outbreaks.

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ADVANCING PANDEMIC PREVENTION AND ONE HEALTH IN THE WHO PANDEMIC AGREEMENT NEGOTIATIONS

Chloé Bâtie[1], Anna Bezruki[2], Alexandra L. Phelan[3], Colin J. Carlson[1]

[1]Department of Epidemiology of Microbial Diseases, Yale University School of Public Health, New Haven, CT, United States, [2]Georgetown University, Washington D.C, DC, United States, [3]Johns Hopkins Center for Health Security, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

For more than a decade, international organizations and the scientific community have urged policymakers to adopt a One Health approach to prevent future pandemics of zoonotic origins. During negotiations for a new international agreement to prevent, prepare and respond to pandemics (the Pandemic Agreement), many WHO Member States pushed to include provisions on prevention and a One Health approach. However, there were difficulties in reaching a consensus on legal obligations, their content, and their prominence in relation to other issues. We aimed to understand how science and politics have shaped the negotiations on these two issues and to make recommendations for future implementation. We transcribed Member States and Relevant Stakeholder (civil society, academia, and industry) statements from open plenary sessions and compiled publicly available draft negotiating texts of the treaty. We thematically coded these documents for prevention and One Health to trace the evolution of these issues in the treaty negotiations. To get a better understanding of what occurred, we conducted interviews with delegates and stakeholders. We found that differences emerged between Global North countries, which generally insisted on expressly including prevention and source of pandemic risk - while Global South countries' comments focused on the consequences, inequities, and practicalities of implementing a One Health approach. The scope of prevention-related activities was raised inconsistently by countries, and spillover drivers were mentioned almost exclusively by stakeholders (e.g., land-use, biodiversity, wildlife trade). Understanding the interests and hesitations of countries - as well as the way the language and focus shifted - in relation to prevention and One Health over the negotiations can contribute to greater understanding of what countries prioritize and comprehend related to prevention and One Health science. This elucidates the broader dynamics of global governance of zoonotic diseases, which can help scientists anticipate evidence gaps, misunderstandings, and areas for scientific communication and advocacy.

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OPTIMAL LONG-ACTING ANTIMALARIAL REGIMENS FOR SEASONAL MALARIA CHEMOPREVENTION: MAPPING REVIEW AND NETWORK META-ANALYSIS

Dhruv Darji, Dhol Samuel Ayuen, Mary Scott, Boris-Enock Zinsou, Adama Kazienga, Prabin Dahal, Philippe J. Guerin, James Watson

Infectious Diseases Data Observatory, University of Oxford, Oxford, United Kingdom

Seasonal Malaria Chemoprevention (SMC) in Sub-Saharan Africa uses a combination of two long-acting antimalarials, sulphadoxine-pyrimethamine plus amodiaquine (SPAQ). However, this combination might not be optimal for all geographic areas, for example, in regions where amodiaquine-based treatment is used, and SP resistance is high. We conducted a systematic review and Bayesian network meta-analysis (NMA) of randomised trials to evaluate the relative efficacies of long-acting antimalarials against uncomplicated and severe malaria for SMC. In total, 18 randomised trials were eligible for the NMA of which 17 were conducted in the Sahel region and one in Uganda. Ranking of the studied regimens based on their reduction in uncomplicated malaria estimated that SPAQ + seasonal malaria vaccination (SMV) with RTS,S vaccine had the greatest efficacy (incidence rate ratio [IRR] of 0.07; 95% CrI 0.06-0.09), followed by the combination of SP + piperaquine (IRR = 0.16; 95% CrI 0.05-0.50); then dihydroartemisinin-piperaquine (IRR = 0.18; 95% CrI 0.15-0.22); SPAQ (IRR = 0.25; 95% CrI 0.24-0.27); and artesunate + amodiaquine (IRR = 0.30; 95% CrI 0.23-0.38). The efficacy of SPAQ + seasonal RTS,S was approximately multiplicative compared with the individual effects of SPAQ (IRR = 0.25; 95% CrI 0.24-0.27) and seasonal RTS,S (IRR = 0.26; 95% CrI 0.21-0.31). For severe malaria, there was no data for piperaquine-based regimens, but SPAQ plus SMV-RTS,S conferred better protection (odds ratio (OR) = 0.12; 95% CrI 0.06-0.28) than SPAQ (OR = 0.42; 95% CrI 0.27-0.65) or SMV-RTS,S (OR = 0.42; 95% CrI 0.22-0.79) when compared to placebo/no chemoprevention. Our findings demonstrate that combining SMC with SMV and exploring alternative regimens to SPAQ could provide greater protection compared to the current strategy. Future trials should directly compare piperaquine-based regimens against SPAQ, and cost-effectiveness models be developed to assess these interventions.

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SAFETY OF MULTIPLE DOSES OF SULFADOXINE PYRIMETHAMINE DURING PERENNIAL MALARIA CHEMOPREVENTION CLUSTER-RANDOMIZED CONTROL TRIAL: AN ASSESSMENT OF ADVERSE AND PASSIVE ADVERSE DRUG EVENTS IN OSUN STATE NIGERIA

Chinazo Nwachi Ujuju[1], Benjamin B. Ilesanmi[1], Mary Adekoya[1], Tolu Kuje[1], Semiu Adebayo Rahman[1], Titus Ojewunmi[1], Anulika Princess Nwosu[2], Uchenna Princess Elemuwa[2], Garba Ahmed Rufai[3], Nnenna Ogbulafor[4], Olusola Oresanya[5], James Kananura Tibenderana[6]

[1]Malaria Consortium, Abuja, Nigeria, [2]National Agency for Food and Drug Administration and Control, Abuja, Nigeria, [3]National Primary Health Care Development Agency, Abuja, Nigeria, [4]National Malaria Elimination Programme, Abuja, Nigeria, [5]Malaria Consortium, Abuja, FC, Nigeria, [6]Malaria Consortium, London, United Kingdom

The Nigeria Perennial Malaria Chemoprevention (PMC) effectiveness study, a cluster-randomized controlled trial, was designed to provide evidence on the impact of PMC on malaria burden and clinical outcomes. Six scheduled doses of sulfadoxine-pyrimethamine (SP) were delivered through routine immunization (RI) and vitamin A supplementation contacts at 10 weeks, 14 weeks, 6 months, 9 months, 12 months, and 15 months of age. Additional pragmatic doses were administered during routine contacts. Given limited evidence on the safety of SP when administered more than five times annually, this study incorporated both passive and active adverse event (AE) monitoring to explore the safety of SP. Passive AE reporting was implemented across intervention and control health facilities in 80 wards in eight local government areas of Osun State. Active AE monitoring was

conducted in a cohort of 1,237 children aged 2-24 months attending four selected health facilities (two intervention, two control). Children were followed up for five consecutive days, after each SP and/or RI administration using phone calls between June 2023 and September 2025 to monitor AEs associated with SP and RI uptake. AEs and demographic characteristics were analyzed using descriptive statistics and multivariable logistic regression. Overall, AE prevalence was low 17.1%. Fever and inconsolable crying were the most frequently reported events, and no serious AEs were observed. AE reporting peaked on the first day following PMC and RI administration, declined thereafter, and was not reported by the fifth day. Children aged 2-4 months had the highest proportion of AEs. Compared with this group, children aged 15-18 months (AOR: 0.140; 95% CI: 0.044-0.442) and 19-24 months (AOR: 0.018; 95% CI: 0.001-0.293) were significantly less likely to experience AEs. Adherence to the four-week PMC dosing interval (AOR: 0.484; 95% CI: 0.266-0.882) and receiving SP alone (AOR: 0.068; 95% CI: 0.013-0.360) were associated with reduced odds of AEs. These findings are consistent with a favourable safety profile for PMC and support repeated SP administration for PMC.

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FACTORS INFLUENCING THE SURVIVORSHIP AND FREQUENT USE OF PERMANET 2.0 LONG-LASTING INSECTICIDAL NETS IN GUATEMALA

Robyn A. Stevenson[1], Norma Padilla[2], Audrey Lenhart[3], **Maria Eugenia Castellanos Reynosa**[1]

[1]James Cook University, Douglas, Australia, [2]Universidad del Valle de Guatemala, Guatemala, Guatemala, [3]U.S. Centers for Disease Control and Prevention (CDC), Atlanta, GA, United States

Malaria causes significant morbidity and mortality worldwide, with ongoing transmission occurring in Latin America. In Guatemala, long-lasting insecticidal nets (LLINs) are a key intervention for malaria prevention and control. Understanding the barriers to LLIN survivorship and use under local conditions is essential to guide malaria control programmes, particularly in Latin American countries, where research on this area remains limited. The aim of this study was to investigate factors influencing survivorship and frequent use of LLINS in a high-risk community in Guatemala. This was a secondary data analysis of three cross-sectional surveys administered at 18-, 24-, and 32-month post-distribution of PermaNet 2.0 in communities from La Gomera, Guatemala (2013-2015). Multivariable Poisson regression models were used to assess factors influencing net survivorship and frequent usage. Adjusted prevalence ratios (aPR) were estimated, along with their corresponding 95% confidence intervals (95% CIs). Factors that influenced LLIN survivorship were the lack of a flush toilet in the household (aPR=1.14, 95% CI 1.05 - 1.23) and the time elapsed since LLIN distribution. Compared to households that received LLINs 18 months before the survey, the prevalence of LLINs present was lower among those surveyed 24 and 32 months after the distribution (aPR=0.89, 95% CI 0.83 - 0.96 and aPR=0.79, 95%CI 0.73 - 0.87, respectively). Seventy-one per cent of the LLINs met the criteria for frequent use. Compared to users who perceived nets should be used year-round, the prevalence of LLIN use was lower among those who believed nets were only needed in the rainy season (aPR 0.38, 95% CI 0.25 - 0.58) or in the dry season (aPR 0.26, 95% CI 0.12 - 0.57). Also, the physical condition of the LLIN was associated with frequent use. This study identifies factors influencing survivorship and the frequency of LLINs use in a Latin American context. It indicates that additional community research, engagement, and education on malaria and the importance of net use are needed before and after distribution campaigns or interventions to ensure effectiveness.

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FEASIBILITY AND ACCEPTABILITY OF AN INTEGRATED IPTP-SP DELIVERY STRATEGY THROUGH SEASONAL MALARIA CHEMOPREVENTION IN ADDITION TO ANTENATAL CARE IN MALI AND BURKINA FASO

Kadiatou Koita[1], Jenna Meredith Hoyt[2], Samba Diarra[3], Adelaide Compaore[4], Mahamadou Dembele[5], Oumou Coulibaly[1], Joel D. Bognini[4], Bihoun Biebo[4], Toussaint Rouamba[4], Dario Scaramuzzi[6], Valerie Briand[7], Halidou Tinto[8], Eve Worrall[9], Kassoum Kayentao[10], Jenny Hill[11]

[1]University of Sciences, Techniques, and Technologies of Bamako, Bamako, Mali, [2]Liverpool, United Kingdom, [3]University of Sciences, Techniques and Technologies of Bamako, Bamako, Mali, [4]Institut de Recherche en sciences de la Santé, Clinical Research Unit of Nanoro, Nanoro, Burkina Faso, [5]Université des Sciences, Techniques et Technologies de Bamako (USTTB), Bamako, Mali, [6]R-Evolution Worldwide Srl Impresa Sociale (REvoWWIS), Naples, Italy, [7]DNDi and Médecins Sans Frontières (MSF), Paris, 75, France, [8]IRSS, Ouagadougou, Burkina Faso, [9]Liverpool Associates in Tropical Health, Liverpool, United Kingdom, [10]Malaria Research & Training Center, Bamako 1805, Mali, [11]Malaria in Pregnancy Consortium, Liverpool, United Kingdom

Malaria threatens ~35 million pregnancies in moderate-to-high-transmission areas in sub-Saharan Africa, yet uptake of intermittent preventive treatment with sulfadoxine-pyrimethamine in pregnancy (IPTp-SP) remains low. The INTEGRATION cluster randomized trial in Mali and Burkina Faso evaluated whether delivering IPTp-SP through seasonal malaria chemoprevention (SMC) alongside antenatal care (ANC) increased coverage of the recommended ≥3 doses. This nested qualitative study assessed the feasibility and acceptability of this strategy. Methods: We conducted in-depth interviews and focus group discussions with health managers, ANC providers, community health workers (CHWs), pregnant and recently delivered women, household heads, and community leaders to explore perceptions of malaria in pregnancy, IPTp-SP, and delivery through SMC+ANC. Transcripts were coded in NVivo-12 and analysed thematically using Bowen's feasibility and Sekhon's acceptability frameworks, applying deductive and inductive approaches. Results: Integrated delivery of IPTp-SP through SMC+ANC was generally feasible and acceptable among CHWs, ANC providers, and managers. Barriers included difficulty locating women, pregnancy concealment, fear of side effects, and challenges tracking community-administered doses. Women, household heads, and community leaders largely accepted the strategy; however, ANC-based delivery was preferred, especially in Burkina Faso, due to greater trust in providers and perceived safety. Suggestions to improve uptake included increased communication, partner involvement, and alternative SP formulations. Scale-up would require early community engagement, adequate CHW resources, and strong local ownership to ensure sustainability. Conclusion: Integrating IPTp-SP into SMC platforms can expand coverage beyond ANC, particularly in underserved areas. Successful scale-up will require addressing implementation barriers, fostering trust in CHWs, enhancing community engagement, and ensuring adequate health system support, including human, financial, and training resources.

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FACTORS ASSOCIATED WITH MALARIA POSITIVITY AMONG CHILDREN UNDER FIVE RECEIVING SEASONAL MALARIA CHEMOPREVENTION: A MULTI-STATE ANALYSIS IN NINE STATES

Olabisi Adebo Ogunmola

Malaria Consortium, FCT, Nigeria

Malaria remains a leading cause of under-five mortality in Nigeria despite high reported coverage of Seasonal Malaria Chemoprevention (SMC). Persistent malaria episodes among SMC recipients suggest implementation, adherence, or contextual barriers that may limit effectiveness. This study assessed factors associated with malaria positivity within 28 days post-SMC among children under five in Nigeria. A secondary

analysis of the 2023 SMC End-of-Round (EoR) survey was conducted across nine states. The primary outcome was caregiver-reported rapid diagnostic test (RDT) positivity within 28 days post-SMC. Independent variables included caregiver characteristics (sex, literacy, education) and child factors (age, sex, first dose ingestion, directly observed therapy for the first dose, and redosing after vomiting). Bivariate associations were assessed using chi-square tests. Variables significant at $p < 0.05$ were included in a multivariable logistics regression to estimate adjusted odds ratios (aORs) and 95% confidence intervals (CIs) for predictors of malaria positivity.Among 11,880 caregiver-child pairs, 93.7% received sulfadoxine-pyrimethamine plus amodiaquine (SPAQ) and 99.4% completed the second and third doses. Caregivers reported fever episodes in 19.5% of children within 28 days post-SMC. Among these, 78.7% sought care at a health facility, where 84.0% were tested using RDT and 81.7% tested positive. Higher odds of malaria positivity were observed among children of male caregivers (aOR: 1.69; 95% CI: 1.46-1.96) and literate caregivers (aOR: 1.32; 95% CI: 1.13-1.54). Complete first dose ingestion was associated with reduced odds of malaria (aOR: 0.61; 95% CI: 0.38-0.99). Children experiencing adverse reactions had threefold higher odds of testing positive (aOR: 3.00; 95% CI: 2.40-3.75). Caregiver and behavioral factors were associated with post-SMC malaria positivity. Unexpectedly, higher malaria positivity was observed among children of literate caregivers. Further investigation is needed to elucidate underlying behavioral, sociodemographic, or pharmacological mechanisms and inform SMC optimizations.

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OPTIMIZING ITN DELIVERY: A COSTEFFECTIVENESS AND COVERAGE ANALYSIS OF DOORTODOOR VERSUS FIXEDPOINT DISTRIBUTION

Orezi Sophronia Adhekoyibo[1], Maureen Okoro[1], Emmanuel Obi[1], Sonachi Ezeiru[1], Nnenna Ogbulafor[2], Suzanne Van Hulle[3]
[1]Catholic Relief Services, Abuja, Nigeria, [2]National Malaria Elimination Programme, Abuja, Nigeria, [3]Catholic Relief Services, Baltimore, MD, United States

Insecticide-treated nets (ITNs) remain one of the most effective malaria prevention interventions, yet large-scale campaigns are resource-intensive. Door-to-door (D2D) delivery maximizes household access but demands substantial personnel and logistics, whereas fixed-point (FP) distribution may reduce operational costs, though its effect on coverage remains insufficient. As malaria programs face increasing financial constraints, understanding the cost-effectiveness and performance tradeoffs between ITN delivery strategies is critical for optimizing campaign design. A comparative cost and coverage analysis across 6 states in Nigeria (Kaduna, Kano, Katsina, Yobe, Taraba, and Niger) was conducted to compare operational costs and programmatic performance of D2D versus FP ITN distributions within integrated SMC/ITN campaigns. Campaign budgets for 2025 integrated SMC/ITN campaigns using FP ITN distribution were compared against budgets for 2022 standalone D2D campaigns using a budget comparison framework. Coverage outcomes on ITN distribution rates and SMC Cycle 1 were compared across the two delivery models. Coverage patterns were assessed across all six states to evaluate consistency of performance. Fixed-point distribution within integrated campaigns was associated with planned cost reductions of 16-24% compared with standalone D2D delivery, driven primarily by consolidation of personnel structures, training, supervision, and logistics. ITN distribution rates under FP delivery ranged from 88-97%, modestly below D2D benchmarks (94-98%), consistent with known differences in uptake dynamics between the two strategies. SMC Cycle 1 coverage remained high across all states under integration (96-99%), comparable to 2024 standalone levels. Cost savings were consistent across states, and integration enabled reallocation of resources toward coordination and monitoring. These findings demonstrate that FP ITN distribution within integrated SMC/ITN campaigns can achieve meaningful cost reductions while maintaining coverage and are more financially sustainable in resource-constrained settings.

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HOW REGULATORY AND MARKET ENVIRONMENTS SHAPE PRIVATE SECTOR SUPPLY CHAINS FOR MALARIA COMMODITIES IN BENIN, CAMEROON AND NIGERIA: INSIGHTS FORM THE ACTWATCH LITE STUDY

Manfred Mario Kokou Accrombessi[1], Keith Esch[2], Katelyn Woolheater[3], Erica Wang[3], Paul Bouanchaud[3]
[1]Population Services International (PSI), Cotonou, Benin, [2]PSI, Washington, DC, United States, [3]Population Services International (PSI), Washington, DC, United States

Background: Malaria continues to pose a significant public health threat in sub-Saharan Africa, the region experiencing most of the world's malaria morbidity and mortality. Private sector outlets play a crucial role in malaria case management. However, understanding of how private sector supply chains function and the challenges they face remains limited. Methods: A multi-country mixed-methods design was used, combining quantitative surveys of 4,723 retail outlets with 66 in-depth interviews conducted among importers and wholesalers between 2023 and 2024 in Benin, Cameroon and Nigeria. Quantitative data described supplier networks and characteristics, while qualitative data explored procurement processes, pricing, distribution models, and challenges. Data were analyzed descriptively and thematically, followed by cross-country comparison. Results: Private supply chains shared a broadly similar structure, including importers, distributors, wholesalers, and retail outlets. However, regulatory and market environments were quite different across countries. Benin operates under regulated pricing and fixed margins, promoting stability but limiting flexibility. Cameroon maintains pharmacist-led importation within a structured yet administratively burdensome system. Nigeria's liberalized market encourages competition and digital innovation but faces limited oversight and significant price volatility. Across all contexts, key challenges included regulatory delays, foreign exchange instability, limited access to credit, weak forecasting, infrastructural barriers, and persistent informal and counterfeit markets. Private sector engagement in rapid diagnostic test distribution remained limited. Conclusion: Strengthening regulatory capacity, improving access to finance, and integrating private actors into national malaria strategies are essential to ensure sustainable access to quality-assured malaria commodities.

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CLIMATE-DRIVEN PREDICTABILITY OF EXTREME DENGUE OUTBREAKS ACROSS SPATIAL AND TEMPORAL SCALES

Valeri N. Vasquez[1], Caroline K. Glidden[2], Erin A. Mordecai[2], Stephan B. Munch[3]
[1]Johns Hopkins University, Baltimore, MD, United States, [2]Stanford University, Stanford, CA, United States, [3]University of California Santa Cruz, Santa Cruz, CA, United States

Dengue is a climate-sensitive disease whose transmission dynamics arise from nonlinear interactions between environmental and epidemiological processes. The extent to which extreme dengue outbreaks are predictable, and under what conditions, remains an open question with important implications for public health preparedness. We present a data-driven framework to evaluate dengue predictability across spatial and temporal scales using Gaussian Process Empirical Dynamic Modeling (GP-EDM), applied to long-term surveillance and climate data from Costa Rica. This approach captures nonlinear, state-dependent relationships between climate and disease dynamics while allowing for comparisons across geographic scales. We find that predictability varies substantially across space. While local dynamics are often heterogeneous and noisy, pooling information across regions reveals shared structure that improves predictive performance, with mean out-of-sample R^2 across provinces ≈ 0.61. Notably, our results show that extreme outbreaks are consistently more predictable than inter-epidemic periods, and that predictability is highest when outbreaks are associated with climate extremes, particularly when temperature and precipitation jointly exceed typical conditions, with mean out-of-sample R^2 increasing by ~0.13 (from ~0.56 to ~0.69). These findings

hold across multiple definitions of climate extremes. These results illustrate that dengue predictability is strongly state-dependent, emerging most clearly under structured environmental forcing. Identifying climate-linked extreme outbreak events as windows of enhanced predictability offers a pathway toward improved early warning systems and more targeted intervention strategies for climate-sensitive disease systems.

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PRENATAL HEAT STRESS AND SMALL VULNERABLE NEWBORNS: EVIDENCE FROM CRADLE COHORT IN BANGLADESH

Farjana Jahan[1], Gabriella Rose Barratt Heitmann[2], ABUL KASHAM MD. SHOAB[3], Abul Hares[1], Mahmudul Hasan[1], Anjuman Ara Rahman[1], Md Mahbubur Rahman[4], Jade Benjamin-Chung[5]

[1]International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Dhaka, Bangladesh, [2]Palo Alto, CA, United States, [3]DHAKA, 13, Bangladesh, [4]Intl Ctr for Diarrhoeal Dis Res, Bangladesh (icddr,b), Dhaka, 13, Bangladesh, [5]Stanford University, Stanford, CA, United States

Prenatal exposure to extreme heat is increasingly recognized as a risk factor for impaired fetal growth, particularly in climatevulnerable regions. However, evidence on cumulative heat exposure and small vulnerable newborns (SVN) a composite measure of fetal vulnerability remains limited in hot and humid South Asian settings. We conducted a prospective observational cohort study of 800 pregnant women in Sirajganj district, northwestern Bangladesh, recruited in early pregnancy and followed through delivery. Maternal characteristics were collected at enrolment, while antenatal care (ANC), delivery history, and birth outcomes were assessed postnatally. Householdspecific daily mean, minimum, and maximum temperatures and Universal Thermal Climate Index (UTCI) were derived from the ERA5 reanalysis dataset. Heat exposure metrics included mean UTCI across pregnancy and proportion of highheat days (maximum temperature 30-35 °C or minimum temperature >28 °C). SVN was defined as preterm birth (<37 completed weeks) or being born too small (birth weight <10th percentile or <2500 g). Associations were examined using prevalence ratios and regression co-efficient adjusting for maternal age, infant sex, socioeconomic assets, ANC visits, and geographic location. Among 780 live births, 35.1% were classified as SVN. Higher mean UTCI during pregnancy was inversely associated with birth weight, with an average reduction of 31 g per 1 °C increase (β = -30.77g; p = 0.024), but not with gestational age. Measures of cumulative heat exposure showed stronger associations, a higher proportion of highheat days during pregnancy was associated with an increased prevalence of SVN (adjusted PR = 5.15; 95% CI: 1.62-16.4). Heatwave exposure (≥2 consecutive days >36 °C) during any trimester was not independently associated with SVN. In conclusion, cumulative prenatal heat exposure was strongly associated with increased risk of small vulnerable newborns in northwestern Bangladesh, highlighting impaired fetal growth as a key pathway linking climaterelated heat stress to adverse perinatal outcomes.

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EXTREME RAINFALL FOLLOWING DRY PERIODS INCREASES ENTERIC PATHOGEN INFECTIONS IN CHILDREN UNDER TWO IN RURAL ECUADOR

Andrea Sosa Moreno[1], Kelsey Jesser[2], Jesse D. Contreras[3], Elinor Artsy[1], Gwenyth O. Lee[4], Gabriel Trueba[5], William Cevallos[6], Dimitrios Gounaridis[1], Karen Levy[2], Joseph Eisenberg[7]

[1]University of Michigan, Ann Arbor, MI, United States, [2]University of Washington, Seattle, WA, United States, [3]Ann Arbor, MI, United States, [4]Rutgers University, New Brunswick, NJ, United States, [5]Univ San Francisco Quito, Quito, Ecuador, [6]Universidad Central del Ecuador, Quito, Ecuador, [7]Univ of Michigan School of Public Health, Ann Arbor, MI, United States

Rainfall can impact the presence of enteric pathogens in the environment and in drinking water sources. Extreme rainfall has been associated with increased diarrhea incidence following dry periods and decreased incidence following wet periods. This phenomenon, known as the concentration-dilution hypothesis, suggests that pathogens accumulate during dry periods and are subsequently flushed into water sources during extreme rainfall events. However, reliance on reported diarrhea captures only a fraction of enteric infections, as many are asymptomatic. Using remotely sensed rainfall data and a prospective birth cohort in rural communities in northwestern Ecuador, we examined the association between extreme rainfall events and enteric pathogen detection in stools samples from children under two years and assessed whether prior rainfall conditions and households' behaviors modified these associations. Stool samples collected at 3, 6, 9, 12, 18 and 24 months (n=2,027) were analyzed by qPCR for 12 molecular enteric pathogen targets. Associations were estimated using generalized estimating equations. The proportion of samples positive for at least one pathogen increased with age, from 54% (183/340) at 3 months to 87% (251/289) at 24 months. Extreme rainfall events in the preceding two weeks were associated with higher odds of detecting at least one pathogen when preceded by dry conditions, compared with periods without extreme rainfall under similar dry conditions (OR: 2.0, 95 CI: 1.2 - 3.4). This association was attenuated among households treating their drinking water (OR: 1.8, 95% CI: 0.9 - 4.0). We observed no statistically significant reduction in the odds detecting at least one pathogen when extreme rainfall followed wet conditions, although the association was in the expected (protective) direction (OR: 0.7, 95% CI: 0.4 - 1.3). These findings are consistent with the concentration-dilution hypothesis, highlighting the role of pathogen accumulation during dry periods and the importance of household water treatment to mitigate the effects of heavy rainfall.

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FLOOD EXPOSURE AND DISRUPTION OF GEOGRAPHIC ACCESS TO ESSENTIAL HEALTH SERVICES IN TANZANIA

Christina Ibrahim Myalla[1], Neema Kafwimi[2], Sosthenes Katende[1], Punam Amratia[3], Susan Fred Rumisha[4]

[1]Ifakara Health Institute, Dar Es Salaam, United Republic of Tanzania, [2]PharmAccess, Dar Es Salaam, United Republic of Tanzania, [3]Perth, WA, Australia, [4]Malaria Atlas Project, University of Oxford, UK, OXFORD, United Kingdom

In Tanzania, floods account for approximately 70% of natural disasters, yet limited evidence exists on how flooding simultaneously affects health facility exposure and access to care. This study assesses flood hazard exposure of essential health facilities and quantifies resulting disruptions to geographic accessibility. Essential health facilities data were compiled, geolocated, and quality-controlled. Flood hazard data from the Joint Research Centre (JRC) Global Flood Awareness System (GloFAS) were used to identify facilities located within or near flood-prone areas and to assess inundation extent. Facilities within a defined buffer of flood-prone areas were classified as vulnerable. Catchment populations were estimated using a travel-time-based allocation approach. A travel-time accessibility model was implemented using a routing engine to compare access to health facilities under baseline and flood conditions. Approximately 11% (1,096) of facilities were located within or near flood-prone areas, with the majority being publicly owned (76%) and dispensaries accounted for the largest proportion (70%). A total of 84 facilities were directly inundated, potentially affecting approximately 950,000 people. Regions with the highest proportion of populations exposed to flood-disrupted health services included Morogoro and Tanga. Preliminary accessibility results indicate that flooding substantially increases travel times and reduces service coverage, with some areas experiencing marked losses in access to nearby facilities and increased reliance on more distant services. These findings demonstrate that flood exposure not only threatens health infrastructure but also disrupts continuity of care by limiting physical access. Integrating flood risk into health system planning is critical to ensure climate-resilient service delivery and safeguard physical access to care during extreme events.

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RATES AND RISK FACTORS OF DENGUE EXPOSURE IN A PEDIATRIC COHORT IN MAKASSAR, INDONESIA

Kavita Dave Coombe[1], Whitney C. Weber[1], Jumriani Ansar[2], Eri Wijaya[2], Ahmad Anakes[2], Ahmad Dzulfikar[2], Najwati Anggraeni[2], Ade Utami[2], Zalsah Puteri Syahrani[2], Auliah Khorunnisa[2], Firda Farid[2], Nirmala Sari[2], Aliyyah Ansariadi[2], Fakhran Farezi Akhyar[2], Ghina U. Anugrah[2], Danil S. Hidayatullah[2], Ansariadi Ansariadi[2], Joelle Rosser[1]

[1]Stanford University, School of Medicine, Palo Alto, CA, United States, [2]Hasanuddin University, Makassar, Indonesia

Climate change and environmental pollution are fueling the rapid increase in incidence and global distribution of Aedes mosquito-transmitted viruses like dengue virus (DENV) and chikungunya virus. Indonesia has some of the highest rates of DENV in the world due to a tropical climate with warm temperatures year-around, heavy rainfall, and flooding. Additionally, Indonesia accumulates some of the highest levels of plastic and trash pollution in their environment, which is a known source of mosquito breeding and disease transmission. Makassar, Indonesia is an environmentally diverse coastal urban city with a large population of residents living in informal settlements, making the population particularly vulnerable to environmental exposures. Makassar is also an area with a high degree of inadequate household water and trash infrastructure, creating compound risk factors for Aedes-borne disease exposure. To understand how climate change influences rates of arbovirus exposure, mothers and their children in Makassar were enrolled into a longitudinal cohort study with blood collection and household exposure surveys of environmental risk factors. We enrolled two separate cohorts of 302 children (2025) and 112 children (2024) aged 1-2 years old across three community health clinics in the Tallo subdistrict. We conducted DENV IgG ELISA assays on serum and detected a 28% DENV seropositivity rate in 2024 and a 48% seropositivity rate in 2025. Our results indicate incredibly high rates of DENV transmission in very young children and have identified trash management as a potential intervention point.

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CONTRASTING EFFECTS OF EL NIÑO FLAVORS ON DENGUE HEMORRHAGIC FEVER EPIDEMICS IN THAILAND REVEALED BY DECADAL VARIABILITY

Jaime Cascante Vega[1], Julian Gottfried[2], Leonardo Souto Ferreira[3], Xavier Rodo[4], Alessandra Gianinni[5], Mercedes Pascual[1]

[1]Center for Genomics and Systems Biology (CGSB), Department of Biology, New York, NY, United States, [2]Department of Biology, Stanford, CA, United States, [3]New York, NY, United States, [4]Climate and Health group, Barcelona Institute for Global Health, Spain, [5]Department of Environmental Studies, New York University, New York, NY, United States

In regions with co-circulation of the 4 DENV serotypes such as Thailand, large epidemics of Dengue Haemorrhagic Fever (DHF) have been attributed to Antibody dependent Enhancement (ADE) as risk of severe disease increases from re-exposure to a heterologous serotype. Another line of research suggests that the El Niño Southern Oscillation (ENSO), the major global driver of interannual climate variability, is a major driver of DHF dynamics. We identify here concomitant changes in temporal patterns of DHF and ENSO at the turn of the century, with an absence of large outbreaks during the first decade across all provinces and an increase in the number of El Niño events of the Central Pacific type (CP), compared to the previously predominant Eastern Pacific events (EP), based on the location of the maximum warming in the Tropical Pacific Ocean. Motivated by this observation, we ask whether changes in ENSO explain the interannual and decadal variability of DHF, with a stochastic SEIRS model whose seasonal transmission rate is the vectorial capacity (VC) as a function of daily temperature. We compare this baseline formulation to extensions that incorporate one or two oceanic El Niño indices (for CP and EP) in the mosquito carrying capacity of the VC. We estimate model parameters with a Sequential Monte Carlo method for likelihood maximization informed by monthly DHF data from Bangkok from 1968 to 2000 and compare models using the Akaike Information Criterion (AIC). With simulation of the selected models from 2000 to 2018, we quantify out-of-sample prediction error using the Continuous Ranked Probability Score (CRPS). We find that the inclusion of two El Niño flavours improves both model fit and out-of-sample predictions, with simulations capturing main features of DHF incidence. EP explains the large seasonal outbreaks of 1987, 1997 and 2015, whereas CP improves prediction of quiescent periods. We conclude that major inter-decadal changes in ENSO influence the interannual variability of DHF in Thailand, suggesting a high degree of sensitivity to Pacific Ocean conditions via teleconnections that drive the population dynamics of the disease.

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MODELING CLIMATE DRIVEN MALARIA RISK: THE ROLE OF ADAPTIVE CAPACITY

Megan Kowalcyk[1], Maxwell Elling[2], Allan Mayaba Mwinde[3], Donnie Mategula[4], James Chirombo[5], James Colborn[6], Baltazar Candrinho[7], Kristopher B. Karnauskas[2], Robert McCann[8], Andrea G. Buchwald[9]

[1]Center for Vaccine Development and Global Health, University of Maryland School of Medicine, Baltimore, MD, United States, [2]College of Arts and Sciences, University of Colorado Boulder, Boulder, CO, United States, [3]Department of Epidemiology and Biostatistics, School of Public Health, University of Zambia, Lusaka, Zambia, [4]Malawi Liverpool wellcome trust, Blantyre, Malawi, [5]Malawi Liverpool Wellcome Trust Clinical Research Programme, Blantyre City, BL, Malawi, [6]Clinton Health Access Initiative, Golden, CO, United States, [7]Ministry of Health, Maputo City, L, Mozambique, [8]University of Maryland SOM, Baltimore, MD, United States, [9]University of Maryland School of Medicine, Baltimore, MD, United States

Climate change is altering the distribution and transmission of malaria through changes to temperature and rainfall patterns. Malaria risk is modified by adaptive capacity factors such as vector control intervention coverage, access to transportation, and education. There is a lack of research on how adaptive capacity factors modify the effects of climate drivers on malaria incidence in sub-Saharan Africa. We employed a generalized linear mixed effects regression modeling framework to assess the relationship between climate variability and malaria incidence, and how adaptive capacity modifies this relationship. Monthly malaria incidence data at the district level were compiled from routine surveillance systems in Malawi, Mozambique, and Zambia from 2010 to 2024. Soil moisture, identified in prior research as a strong predictor of malaria risk in Malawi, was used as the primary climate exposure variable. Monthly district-level soil moisture data were obtained from the Gravity Recovery and Climate Experiment. To account for environmental changes, annual land cover data were sourced from the Food and Agriculture Organization. Adaptive capacity data was obtained from demographic and health surveys and malaria indicator surveys conducted in the region between 2010 to 2024 with linear extrapolation to obtain yearly estimates. We found that soil moisture has a non-linear significant association with malaria incidence in all three countries. In Malawi we found that risk of malaria due to soil moisture was significantly decreased as the proportion of children under the age of 5 who slept under a bed net in each district increased. Similarly, the proportion of households with electricity in a district significantly decreases the association between soil moisture and malaria incidence. Overall, these results demonstrate the importance of incorporating adaptive capacity factors into models assessing the effect of climate change on future malaria risk. Additionally, successful implementation of interventions to increase adaptive capacity, such as bed net use, can increase resiliency to the impact of climate change on malaria in the region.

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SYSTEMATIC REVIEW AND SECONDARY DATA ANALYSIS TO ASSESS THE EFFECT OF CLIMATE CHANGE ON MALARIA IN PREGNANCY IN MALARIA-ENDEMIC REGIONS

Tianna Felder[1], Tiarra Felder[1], Elizabeth Wagman[2], Andrea Buchwald[3], Sarah Boudova[4]

[1]University of Maryland, Baltimore, School of Medicine, Department of Epidemiology and Public Health, Baltimore, MD, United States, [2]Sidney Kimmel Medical College at Thomas Jefferson University, Obstetrics and Gynecology, Philadephia, PA, United States, [3]University of Maryland School of Medicine, Center for Vaccine Development and Global Health; Liverpool School of Tropical Medicine, Department of Clinical Sciences, Baltimore, MD, United States, [4]University of Illinois Chicago, Assistant Professor of Maternal-Fetal Medicine and Director of Perinatal Global Health, Chicago, IL, United States

Plasmodium falciparum malaria in pregnancy (MiP) is a significant cause of maternal and child morbidity and mortality. Climate change influences the spread of malaria through temperature, rainfall, humidity, and the ecology of malaria vectors. There is substantial research on the relationship between climate and malaria, however there is a gap in the literature concerning how climate is related to malaria among pregnant women as a distinct high-risk group. We conducted a systematic review under a registered protocol (CRD420251147477) to synthesize primary data on *P. falciparum* MiP. Cohort studies and randomized controlled trials (RCT) published between the years 1980 and 2024 were included. We excluded studies which did not report on pregnant groups or MiP, could not be used to estimate incidence or prevalence of MiP, and non-primary sources. Following a comprehensive search on PubMed, we identified 676 peer-reviewed articles, 128 duplicates were removed. 285/548 abstracts were excluded for irrelevance, 35/263 full-text peer-reviewed articles were excluded due to not meeting inclusion criteria or lacking sufficient data quality. Of 228 relevant peer-reviewed articles, 144 studies had data on distinct cohorts of pregnant women, and 38/228 friend studies were excluded. This dataset formed the basis for subsequent data extraction and analysis for further contacting authors for the datasets. The total combined sample size of these parent studies is 252,426 participants in various endemic regions including Africa Region (n=96 studies), South-East Asia Region (n=28 studies), Region of Americas (n=8 studies), and multi-regional (n=12 studies). There were 44 RCTs/clinical trials (131,200 participants) and 100 observational studies, including cohort, cross-sectional, and case-control studies (121,226 participants). Individual-level data extraction is ongoing, and a final dataset will be merged with climate data from the University of California Climate Hazards Center (CHIRPS and CHIRTS) to assess the relationship between climate variability and MiP.

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A SCOPING REVIEW OF ARTIFICIAL INTELLIGENCE FOR CLIMATE-DRIVEN NEGLECTED TROPICAL DISEASES RISK MODELLING IN AFRICA: EVIDENCE, GAPS, AND IMPLICATIONS FOR WHO 2030 ELIMINATION TARGETS

Jolly Akor Thomas, Igdaliah Jesuloluwa Otitoola, Sidiq Ayomide Moshood, Joseph Chizaram Ezima

University of Ibadan, Ibadan, Nigeria

Neglected tropical diseases (NTDs) account for about 57 million disability-adjusted life years lost annually in sub-Saharan Africa. Climate change is reshaping NTD transmission dynamics. Artificial intelligence (AI) offers new capabilities for modelling and forecasting disease risk. However, the extent to which AI is being applied at the climate-NTD interface in Africa, and their implications for achieving the WHO 2030 elimination targets, remains unclear. This scoping review mapped the scope and nature of evidence on the application of AI to model climate-driven NTD risk in Africa and identified key evidence gaps for policy and control. This scoping review followed the Arksey and O'Malley framework and PRISMA-ScR guidelines (OSF DOI: https://doi.org/10.17605/OSF.IO/P8DKA). PubMed, Scopus, Web of Science, Embase, Global Health, and WHO IRIS were searched for studies published between 2000 and 2025 that applied AI to model climate-driven NTD risk in Africa. 24 studies met the inclusion criteria, spanning nine NTD categories across 17 countries, with evidence geographically concentrated in West Africa. Schistosomiasis was the most studied NTD (41.7%). No eligible studies were identified for trachoma, leprosy, yaws, or mycetoma. Random Forest (58.3%) and MaxEnt niche modelling (25.0%) were the predominant algorithms. Temperature (79.2%) and precipitation (75.0%) were the most commonly used predictors. Model performance was high (AUC: 0.71-0.96; R^2: 0.62-0.93). Only 2 studies (8.3%) incorporated future climate projections. No study reported the use of formal explainable AI frameworks or co-design with national NTD program managers. Only 2 studies (8.3%) received African institutional funding. Despite strong predictive performance, this evidence base is characterized by poor NTD coverage, near-zero future climate modelling, and absent explainability standards. African-led model development anchored in local data, adoption of explainable AI standards, and embedding of climate-AI forecasting into national NTD control programs are key to climate-resilient progress toward the WHO 2030 elimination targets.

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AUTOMATED B-LINE DETECTION AND LOCALIZATION IN LUNG ULTRASOUND USING UNSUPERVISED ANOMALY DETECTION TO SUPPORT PULMONARY ASSESSMENT IN MALARIA-ENDEMIC SETTINGS

Yanlin Wu[1], Tiffany Fong[2], Patrick Tshilab[3], Jean-Bertin Bukasa Kabuya[4], Matthew M. Ippolito[2], Muyinatu Bell[1]

[1]Johns Hopkins University, Baltimore, MD, United States, [2]Johns Hopkins University School of Medicine, Baltimore, MD, United States, [3]Saint Paul's Mission General Hospital, Luapula, Zambia, [4]National Health Research and Training Institute, Copperbelt, Zambia

Pulmonary involvement in malaria ranges from subclinical increases in lung density to pulmonary edema and acute respiratory distress syndrome. Prior lung ultrasound studies have shown that B-lines are more frequent in patients with uncomplicated malaria than in healthy controls and decrease after antimalarial treatment. B-line patterns in malaria have also been associated with dyspnea, crepitations, lower oxygen saturation, and higher parasite burden. In resource-limited settings, reliable pulmonary assessment by point-of-care ultrasound is constrained by operator dependence and limited interpretive expertise. We therefore developed an automated framework for B-line assessment in lung ultrasound images that integrates image-level classification with unsupervised anomaly detection to estimate B-line number and location. We analyzed 4,282 lung ultrasound images from 55 children with severe malaria, comprising 883 images from 29 B-line-positive regions and 3,399 from 176 regions without B-lines. A classifier was trained to distinguish images with and without B-lines. On a held-out test set, the classifier achieved an area under the receiver operating characteristic curve of 0.9946, accuracy of 0.9642, F1 score of 0.9474, sensitivity of 0.9439, and specificity of 0.9722. Images classified as B-line positive were further then passed to an unsupervised anomaly detection framework that used reconstruction residuals between each input image and its pseudo-normal estimate to infer B-line number and location, without requiring pixel-level B-line annotations for training. Preliminary visualizations showed plausible localization patterns in B-line-positive images, supporting the potential of ultrasound anomaly-based analysis as a reproducible imaging biomarker. This approach may support scalable assessment of malaria-associated pulmonary involvement, including early interstitial fluid accumulation or reduced lung aeration, in settings where expert lung ultrasound interpretation is limited.

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BEYOND PREVALENCE SURVEILLANCE: REAL-TIME INFERENCE OF MALARIA TRANSMISSION, IMMUNITY, AND INTERVENTION IMPACTS USING DEEP LEARNING SURROGATE

Olatunde E. Ibrahim, Joseph Hicks, Peter Winskill, Patrick Walker
Imperial College London, London, United Kingdom

Precise malaria control requires timely estimates of transmission intensity and disease burden, yet standard methods for inferring key epidemiological parameters are computationally intensive and slow. This research bridges this gap by developing machine learning (ML) surrogate trained on outputs of a mechanistic malaria model, parameterized by antennal care (ANC) derived inputs, to provide instantaneous population level epidemiological insights. Using these data, we developed a multi-scale convolutional temporal encoder to perform inverse inference, mapping temporal sequences of infection prevalence and intervention coverage directly to latent malaria indicators, including entomological inoculation rate (EIR), functional immunity, and clinical incidence. The surrogate achieves high predictive accuracy across varying endemicity levels, matching the outputs of traditional Bayesian inference methods at a fraction of the computational cost. Notably, the model captures non-linear relationships between prevalence and burden, allowing for the rapid projection of time varying intervention - such as the impact of seasonal malaria chemoprevention (SMC) or vaccine without requiring high-performance computing. This framework enables the integration of sophisticated modelling into routine surveillance systems like District Health Information System (DHIS2). By harnessing the growing availability of ANC-based surveillance and the speed of deep learning, this work empowers National Malaria Control Programmes (NMCPs) with a scalable, adaptive decision-support system for more efficient resource allocation and real-time situational awareness. Ongoing work involves developing automated diagnostic layers to detect, flag and mitigate the impact of out-of-sample prevalence data. By addressing reporting anomalies, we ensure the framework remains resilient to potential reporting inconsistencies in routine surveillance systems.

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WHAT DEFINES A GENE DELETION? DISCREPANT RESULTS ACROSS MOLECULAR METHODS REVEAL COMPLEX *PFHRP2/3* PATTERNS IN *PLASMODIUM FALCIPARUM* FROM THE BRAZILIAN AMAZON

Tuany Rodrigues[1], Keithy Wenny Plaster Lima[2], Michelle Oliveira-Silva[2], Dhelio Batista Pereira[2], Alysson Henrique Urbanski[3], Tiago Minuzzi[3], Johanna Helena Kattenberg[4], Anna Rosanas Urgell[4], Alexandre Costa[1]
[1]Fiocruz Parana, Curitiba, PR, Brazil, [2]CEPEM, Porto Velho, RO, Brazil, [3]Centro de Saúde Pública de Precisão - CSPP, Curitiba, PR, Brazil, [4]Institute of Tropical Medicine, Antwerpen, Belgium

Molecular surveillance is essential to support malaria control and elimination. Rapid diagnostic tests (RDTs), widely used in remote settings, rely on HRP2 detection, making early and accurate diagnosis possible. However, deletions in *pfhrp2* and *pfhrp3* genes can lead to false-negative results. Reported prevalence of these deletions varies widely across studies, raising concerns about how gene deletions are defined and detected using different molecular approaches. In this study, we investigated discrepancies in *pfhrp2/3* deletion detection across 6 molecular assays and explored deletion patterns in *Plasmodium falciparum* from Rondônia, Brazil. Initially, 63 samples were analyzed using multiple PCR-based approaches, including two conventional PCR (cPCR), three qPCR assays, and droplet digital PCR (ddPCR). Subsequently, 38 samples were analyzed using next-generation sequencing (NGS) with the AmpliSeq platform. Deletions were inferred based on normalized read depth (DESeq2) and supported by a CNV-based approach using read count data. PCR-based methods showed discrepant results across all approaches. For *pfhrp2*, deletion rates varied among protocols, ranging from 11.1-23.8% for cPCR and 0-9.5% for qPCR, while ddPCR did not detect deletions. For *pfhrp3*, deletion rates ranged from 11.1-71.4% among qPCR protocols, whereas ddPCR detected deletions in 95.2% of samples. In contrast, AmpliSeq data revealed a clear genomic pattern. Complete deletion of *pfhrp2* was rare (2.9%, 1/35), while *pfhrp3* showed high prevalence of deletion across samples (100%, 35/35), with absence of gene coverage consistent with gene loss. For *pfhrp2*, coverage profiles were variable, with reduced coverage in specific regions and CNV patterns consistent with partial or structurally complex gene loss rather than complete deletion. Our results clearly show that detection of *pfhrp2/pfhrp3* deletions is highly dependent on the molecular protocol used. These findings highlight the need for standardized approaches and support the use of NGS-based methods for accurate molecular surveillance, with direct implications for malaria control strategies.

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DETECTION OF FALSE NEGATIVE RDT RESULTS DUE TO PFHRP2 DELETION AMONG SYMPTOMATIC MALARIA INFECTIONS, AT THE ISIOLO REGIONAL HOSPITAL, ISIOLO COUNTY, KENYA

Christopher Ekuttan[1], Milton Obilo[1], John Lugonzo[1], Juliana Munyao[1], Maurine Atieno Mwalo[2], Edwin Mwakio[3], Gladys Chebet Chemwor[4], Jackline Akinyi Juma[5], Sharon Kiptoo[1], Teresia Kimundui[6], Redemptah Yeda[7], Raphael Onyango Okoth[4], Benjamin Opot[8], Dennis Wekesa Juma[9], Robert C. Haynes[10], Luis Pow Sang[11], Bernhards Ogutu[12], Hoseah Akala[7]
[1]Ministry of Defence, Kenya Defence Forces, Nairobi, Kenya, [2]USAMRD-K, Kisumu, Kenya, [3]Kenya Medical Research Institution, Kisumu, Kenya, [4]HJFMRI, Kisumu, Kenya, [5]KEMRI-WRP, Nairobi, Kenya, [6]2Walter Reed Army Institute of Research -Africa/Kenya Medical Research Institute (KEMRI)., Kisumu, Kenya, [7]USAMRU-K, Kisumu, Kenya, [8]United States Army Medical Research Unit - Kenya, Nairobi, Kenya, [9]United States Army Medical Research Directorate-Kenya, Kisumu, Kenya, [10]Walter Reed Army Institute of Research-Africa., Kisumu, Kenya, [11]Walter Reed Army Institute of Research-Africa., Kisumu, Kenya, [12]KEMRI- Walter Reed Project, Nairobi, United States

PfHRP2-based rapid diagnostic tests (RDTs) are widely used in endemic regions due to affordability and ease of use in settings lacking microscopy. However, parasites with pfhrp2/3 deletions are not detected, leading to false-negative results and reduced diagnostic accuracy. We assessed the frequency of false-negative PfHRP2 RDT results among symptomatic malaria patients at Isiolo Regional Hospital, Kenya. Blood samples were collected from individuals ≥6 months presenting with malaria symptoms. Diagnosis was performed using microscopy, PfHRP2 and PAN mRDTs, and PCR. *P. falciparum*–positive samples were sequenced to determine pfhrp2/3 deletion profiles. Overall, 96.7% (29/30) of samples were positive by microscopy, PAN mRDT, and PCR, compared to 90% (27/30) by PfHRP2 RDT. *P. falciparum* was detected in 96.6% (28/29). P. ovale curtisi occurred in 6.9% (2/29), including one mono-infection that tested PfHRP2-negative. Control gene amplification was successful in 29/30 samples. HRP2 was intact in 96.4% (27/28), with one confirmed deletion (3.6%). HRP3 deletions were observed in 85.7% (24/28). The HRP2-negative case showed dual HRP2/3 deletion and was non-reactive on PfHRP2 RDT. This represents the first report of symptomatic *P. falciparum* with false-negative PfHRP2 diagnosis due to double deletion in Isiolo. Findings align with national surveys showing high HRP3 deletion prevalence in Northeastern Kenya. In conclusion, False-negative PfHRP2 results occurred in 6.8% of infections (3.4% HRP2/3 deletions; 3.4% P. ovale mono-infection), exceeding the WHO 5% threshold. This poses a significant threat to diagnostic reliability and supports reconsideration of HRP2-based RDT use in the region. Deployment of pan-species RDTs and strengthened prophylaxis targeting hypnozoite-forming species are recommended.

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PREVALENCE OF *PLAMODIUM FALCIPARUM* SUBPATENT INFECTIONS AMONG ASYMPTOMATIC INDIVIDUALS IN FIVE REGIONS OF MAINLAND TANZANIA. A COMMUNITY-BASED CROSS-SECTIONAL STUDY, 2023

Hussein J. Semboja[1], Rule Budodo[1], Angelina Kisambale[1], Salehe Mandai[1], Dativa Pereus[2], Catherine Bakari[1], Baraka W. Mbuba[3], Gervas Chacha[4], Happyness Mwailunga[1], Laurencia S. Tangawizi[1], Gideoni I. Maulidi[1], Ninja S. Bisaga[4], Daniel Protasy Challe[5], Daniel A. Petro[6], Rashid A. Madebe[4], Solomon Mwakasungula[1], Daniel Mbwambo[7], Sijenunu Aaron[7], Abdallah Lusasi[7], Samuel Lazaro[7], Stella Kajange[8], Celine I. Mandara[4], Misago D. Seth[4], Deus S. Ishengoma[1]

[1]Ifakara Health Institute, Dar Es Salaam, United Republic of Tanzania, [2]Muhimbili University of Health and Allied Science, Dar Es Salaam, United Republic of Tanzania, [3]Nelson Mandela African Institution of Science and Technology, Arusha, United Republic of Tanzania, [4]National Institute for Medical Research, Dar Es Salaam, United Republic of Tanzania, [5]National Institute for Medical Research, Tanga, United Republic of Tanzania, [6]University of Dar es Salaam, Dar Es Salaam, United Republic of Tanzania, [7]National Malaria Control Program, Dodoma, United Republic of Tanzania, [8]Prime Minister's Office, Regional Administration and Local Government, Dodoma, United Republic of Tanzania

Asymptomatic individuals in endemic communities often harbor patent or subpatent malaria infections but rarely seek care, resulting in their exclusion from routine surveillance systems. These individuals may carry transmissible gametocytes, thereby sustaining community-level transmission, and can also harbor molecular markers associated with antimalarial drug resistance Data from a community-based cross-sectional survey conducted between July and August 2023 were used to assess the prevalence of *Plasmodium falciparum* subpatent infections across five regions with varying transmission intensities in Mainland Tanzania. The survey included regions from high, moderate and very low transmission strata. Participants aged ≥6 months were tested for malaria using rapid diagnostic tests (RDTs), and dried blood spot (DBS) samples were collected (n = 9,010). DNA from selected RDT-negative samples (n = 2,899) was extracted using the Tween-Chelex method and analyzed by 18S rRNA quantitative PCR (qPCR) to detect subpatent infections. Of 2,899 samples, 191 (6.6%;95% CI: 5.7-7.6) were positive by qPCR. Prevalence was also significantly higher among adults (aged >15+ years, with 7.5%), followed by school children (aged 5 - <15 years, with 6.1%) and the lowest was in under-fives (3.5%, p = 0.009). The prevalence and odds of subpatent infections were significantly higher regions with very low transmission intensities (prevalence = 47.5%, 95% CI: 35.3 - 60.0; aORs =1.00), compared to regions with moderate (6.4%, 95% CI: 3.1-12.6; aOR = 0.08) and high transmission (5.7%, 95% CI: 4.9 - 6.7'; aOR = 0.07). These findings indicate that subpatent infections caused by *P. falciparum* are most common in adults and in regions with very low transmission intensities. This underscores the importance of implementing more sensitive, community-based surveillance to detect asymptomatic carriers sustaining malaria transmission.

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OPTIMIZING SCREEN-AND-TREAT STRATEGIES USING A NEAR POINT OF CARE MOLECULAR TEST TO DETECT SUB-PATENT MALARIA INFECTIONS IN PREGNANT WOMEN IN ZAMBIA: PRELIMINARY RESULTS.

Jean-Bertin Bukasa Kabuya[1], Ivana Pennisi[2], Sydney Bukasa Mwanza[1], Ephraim Chikwanda[1], Prudence Hamade[3], Halidou Tinto[4], Aubrey J. Cunnington[5], Christine Manyando[1], Jean-Pierre Van geertruyden[6], Jesus Rodriguez-Manzano[2]

[1]National Health Research and Training Institute, Ndola, Zambia, [2]Department of Infectious Disease, Imperial College, London, United Kingdom, [3]Malaria Consortium, London, United Kingdom, [4]IRSS Centre Muraz, Ouagadougou, Burkina Faso, [5]Section of Paediatric Infectious Disease, Department of Infectious Disease, Imperial College, London, United Kingdom, [6]Global Health Institute, University of Antwerp, Antwerp, Belgium

In areas of stable malaria transmission, *Plasmodium falciparum* malaria in pregnancy (MIP) is often asymptomatic and submicroscopic due to placental sequestration, yet it remains associated with adverse pregnancy outcomes. Sulfadoxine–pyrimethamine (SP), used for intermittent presumptive treatment in pregnancy (IPTp), reduces these complications; however, increasing resistance threatens its effectiveness. Screen-and-treat (SAT) strategies represent potential alternatives but are limited by the poor sensitivity of RDTs in detecting subpatent MIP infections. Highly sensitive point-of-care (POC) diagnostics are therefore needed. PCR is highly sensitive but not field-friendly, whereas loop-mediated isothermal amplification (LAMP) can be integrated into portable devices and be used in field settings. Within a clinical trial comparing IPTp-SP with a hybrid strategy (SAT plus IPTp-SP) among malaria asymptomatic pregnant mothers in Nchelenge district in northern Zambia, we evaluated the diagnostic accuracy of a sample-to-result LAMP-based device (Dragonfly). Participants in the hybrid arm were tested at their first antenatal visit using RDT, microscopy, and Dragonfly; dried blood spot (DBS) were collected for qPCR. Participants received dihydroartemisinin–piperaquine if RDT-positive or SP if RDT-negative. A total of 137 samples were analysed. Dragonfly positivity rate was 53% (95% CI: 45–62%), compared with 60% (95% CI: 51–68%) by DBS-qPCR, 41% (95% CI: 33–49%) by RDT, and 36% (95% CI: 29–45%) by microscopy. Sensitivity and specificity were 88% (95% CI: 79–94%) and 98% (95% CI: 92–100%) for Dragonfly, 66% (95%: 56-76%) and 96% (CI: 86-100%) for RDT, and 61% (95% CI: 50-72%) and 100% (95% CI: 94-100%) for microscopy. Positive and negative predictive values for Dragonfly were 99% (95% CI: 92–100%) and 84% (95% CI: 73–92%). Compared with RDT, Dragonfly detected 22% more qPCR-positive infections, corresponding to a 33% relative increase (95% CI: 25–41%; McNemar p < 0.001) . These findings support its use in SAT-based MIP prevention strategies, including intermittent screening in pregnancy and treatment and hybrid approaches.

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CHALLENGES IN MALARIA DIAGNOSIS AND TREATMENT IN KINSHASA PROVINCE, DEMOCRATIC REPUBLIC OF THE CONGO

Ruthly François-Zafka[1], Daniel Westreich[1], Kristin Banek[2], Melchior Mwandagalirwa Kashamuka[3], Samuel J. White[1], Joseph A. Bala[3], Marthe Nkalani[3], Georges Kihuma[3], Joseph A. Losoma[3], Georges E. Mahilu[3], Kyaw Thwai[1], Kokolomami Jack[3], Michael Emch[1], Jessie Edwards[1], Jonathan J. Juliano[1], Antoinette Tshefu Kitoto[3], Jonathan Parr[1]

[1]University of North Carolina at Chapel Hill, Chapel Hill, NC, United States, [2]UNC Institute for Global Health & Infectious Diseases, Chapel Hill, NC, United States, [3]University of Kinshasa, Kinshasa, Democratic Republic of the Congo

Effective malaria case management relies on accurate diagnosis and appropriate treatment. However, rapid diagnostic test (RDT) performance varies, and presumptive treatment remains common. We quantified malaria misdiagnosis and inappropriate treatment and assessed adherence to treatment guidelines in a high-burden setting. We leveraged samples collected during a cohort study of children and adults from 2015 to 2022 across seven sites in Kinshasa Province, Democratic Republic of the Congo (DRC). We estimated the cumulative incidence and prevalence of false-positive and false-negative RDT results using PCR as the gold standard and identified correlates of misdiagnosis and inappropriate treatment using Kaplan Meier and generalized estimating equations. Among 2,269 participants, the one-year cumulative incidences of false-positive and false-negative RDTs were 23.1% and 17.8%, respectively; by seven years, these rose to 77.7% and 83.4%. Misdiagnosis (false-positive or -negative RDT) was associated with age, recent treatment, transmission intensity, and parasite density -factors that could inform refinements to treatment guidelines. About 58% of RDT-negative participants received antimalarial treatment at a clinic visit, while 4% of RDT-positive participants

did not. Fever significantly reduced adherence to test-and-treat guidelines. Most participants experienced malaria misdiagnosis by RDT over the course of the study, underscoring the limitations of currently used RDTs and opportunities for improved clinical decision-making. While RDTs remain essential for malaria case management, clinicians should consider contextual factors when interpreting results. Efforts to improve adherence to guidelines, which require a positive diagnostic test result before treatment, and management of non-malarial febrile illness are needed in the DRC and similar high-burden settings.

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STRENGTHENING MALARIA DIAGNOSIS AND CASE MANAGEMENT THROUGH TRAINING AND SUPPORTIVE SUPERVISION: EXPERIENCE FROM THE BIOKO ISLAND MALARIA ELIMINATION PROJECT

IniAbasi Nglass[1], Adrian May[1], Elizabeth Lucas Nyakarungu[1], Maria Consuelo Oki Eburi[1], Matilde Riloha Rivas[2], Julie Guadalupe Buekens[3], Wonder Philip Phiri[1], Carlos Alberto Guerra[4], Guillermo A. Garcia[3]

[1]MCD Global Health, Malabo, Equatorial Guinea, [2]National Malaria Control Program, Malabo, Equatorial Guinea, [3]MCD Global Health, Hallowell, ME, United States, [4]MCD Global Health, Quito, Ecuador

Accurate diagnosis and effective case management are vital for malaria elimination. Since 2022, the Bioko Island Malaria Elimination Project (BIMEP), in collaboration with Equatorial Guinea's Ministry of Health and National Malaria Control Program (NMCP), has implemented Outreach Training and Supportive Supervision (OTSS) alongside capacity-building interventions and refresher training. Between 2022 and 2024, 147 health workers (clinicians and laboratory technicians) participated in refresher training. Training assessments included pre/post-tests, WHO-standard microscopy evaluations, and quarterly OTSS digital checklists. Theoretical average scores rose from 68% to 84% post-training. While microscopy competency in parasite detection averaged 68%, only 10% achieved competency in parasite counting. However, OTSS scores demonstrated steady growth: laboratory performance (parasite detection and counting) increased from 63% (2023) to 71% (2026), and clinical management scores rose from 51% to 83%. As part of the training structures, between 2023 and 2024, 48 potential district trainers were evaluated; 29 (60%) met the ≥80% facilitation threshold and received mentorship. Consequently, 16 trainers were certified in 2026. BIMEP's structured training, OTSS, and data reviews have significantly improved competency in malaria diagnosis and management. To ensure sustainability, investing in decentralized district-level trainer capacity remains a strategic priority.

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SUBSIDY WITHDRAWAL IMPACT: COMPARATIVE ANALYSIS OF ARTEMISININ-BASED COMBINATIONTHERAPIES (ACTS) AND MALARIA RAPID DIAGNOSTIC TESTS (MRDT MARKETS IN NIGERIA'S PRIVATE SECTOR (2018 & 2024)

Segun Emmanuel Ibitoye[1], James Soujah Abu[1], Dawit Getachew[1], **Olusola Oresanya**[1], Kolawole Maxwell[1], Emmanuel Shekarau[2], Nnenna Ogbulafor[2]

[1]Malaria Consortium, Nigeria, Nigeria, [2]National Malaria Elimination Programme, Abuja, Nigeria

The private sector plays a central role in malaria control in Nigeria. However, gaps remain in demand, supply chain structures, the use of locally manufactured brands of artemisinin-based combination therapies (ACTs), and the market share of malaria rapid diagnostic tests (mRDTs). This study assessed changes in availability, affordability, use, and perceptions of ACTs and mRDTs between 2018 and 2024. Outlet surveys of private pharmacies and Proprietary and Patent Medicine Vendors (PPMVs) were conducted across six geopolitical zones; 695 outlets were sampled in 2018 and 1,168 in 2024 via cluster sampling. Descriptive, chi-square, and regression analyses were conducted with statistical significance defined as $p \leq 0.05$ and estimates are presented with 95% confidence intervals (CIs). By 2024, ACT markets had grown, while mRDT uptake stagnated. Formerly subsidized ACT brands under the Affordable Medicines Facility-malaria (AMFm) (Lumartem, Combisunate) declined, replaced by Amatem Forte (49%), Lonart (49%), and Coartem (42%). Southern states had higher Amatem Forte availability (2018: 50%; 2024: 66%) than northern states (2018: 33%; 2024: 39%). Self-reported encounters with counterfeit ACTs declined sharply (2018: 68% vs. 2024: 11%). After adjusting for inflation in 2024, the ACT price volatility persisted, driven by supply chain inefficiencies, product scarcity, and reliance on multiple distribution channels, with urban pharmacies charging more than rural PPMVs. mRDT availability remained low: 22% of pharmacies and 14% of PPMVs stocked mRDTs in 2018, compared with 21% and 16% in 2024. Logistic regression showed PPMVs were more likely than pharmacies to encounter counterfeit ACTs (AOR 2.21; 95% CI: 1.49 to 3.27; $p < 0.001$). Persistent awareness, supply, and trust deficits hindered mRDT adoption, leaving the public sector as the diagnostic backbone while the private sector drove ACT access. Targeted interventions to strengthen supply chains, enforce test-and-treat policies, regulate pricing, and train outlets may help to balance ACT and mRDT demand and advance equitable malaria control.

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MOSQUITO SPECIES DIVERSITY, HOST ASSOCIATIONS, AND THE *PLASMODIUM* COMMUNITY IN SOUTHERN ZAMBIA

Kochelani Saili[1], Raymond W. Gellner[1], Alexander Urlaub[1], Abigail A. Hinton[1], Corbin J. Puckett[1], Limonty M. Simubali[2], Gift Mwaanga[2], Nathan D. Burkett-Cadena[1], Lawrence E. Reeves[1]

[1]University of Florida Medical Entomology Laboratory, Vero Beach, FL, United States, [2]Macha Research Trust, Choma, Zambia

Mosquito blood meals are a valuable source for identifying host species and circulating pathogens. This study assessed mosquito species diversity in Southern Province, Zambia, examined host association of mosquito species through DNA-based barcoding analysis, and identified potential pathogens from different vertebrate blood meals. Mosquitoes were collected outdoors in four districts (February-March 2025) using battery-powered wide diameter aspirators. Blood-fed specimens were morphologically identified. The engorged abdomen of each blood-fed mosquito was crushed on Whatman Flinders Technology Associates card, thus preserving DNA contained within the mosquito abdomen. DNA was extracted using the sodium hydroxide-Tris method and amplified using hierarchical PCRs targeting the vertebrate cytochrome c oxidase subunit I gene. Amplicons were Sanger sequenced and resulting sequences were compared to publicly accessible DNA sequence databases for species identification. Haemosporidian parasites (*Plasmodium, Leucocytozoon*) were detected using a nested PCR. A total of 343 blood-fed mosquitoes representing 46 species were collected, dominated by *Culex antennatus* (35.2%). Other species included *Aedes leesoni*, *Ae. mcintoshi*, *Anopheles gambiae s.l., Cx. bitaeniorhynchus* and species from the genera *Ficalbia, Lutzia, Mimomyia, Uranotaenia,*and *Aedeomyia.* Of these, 278 successfully yielded amplicons, identifying 112 unique host species: 21 bird, 14 mammal, six amphibian, and two reptile species. *Culex* mosquitoes showed the broadest feeding range (35 host species; Simpson diversity index=0.49), while *Aedes* fed mainly on mammals (11 host species: Simpson diversity index=0.63). Haemosporidian DNA was detected in 19 mosquitoes, including *Plasmodium juxtanucleare* and *Haemoproteus macrovacuolatus*, associated with *Cx. antennatus, Cx. bitaeniorhynchus, Cx. quinquefasciatus*, and *Ae. argenteopunctus.* These findings highlight *Culex* mosquitoes as generalist feeders capable of linking wildlife, livestock, and humans, potentially facilitating cross-species pathogen transmission and spillover.

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MOSQUITO SPECIES COMPOSITION, DIVERSITY, AND ARBOVIRUS PREVALENCE ACROSS DIFFERENT HABITAT TYPES IN THE GAMBIA

Muhammed M Camara[1], Dr. Gillian Eastwood[1], DR. Marta Moreno[2], Dr. Diawo Diallo[3], Harouna Dit Massire Soumare[4], Ebrima A. Jawara[5]

[1]Virginia Tech University, Blacksburg, VA, United States, [2]London School of Hygiene and Tropical Medicine, London, United Kingdom, [3]Institute Pasteur Dakar, Senegal, Dakar, Senegal, [4]Medical Research Council The Gambia@ LSHTM, Fajara, Gambia, [5]Medical Research Council, The Gambia@ LSHTM, Fajara, Gambia

Mosquito-borne arboviruses pose a significant One Health challenge in sub-Saharan Africa, yet surveillance remains limited in many countries, including The Gambia. Although arbovirus circulation has been documented in neighboring West Africa, the status of arboviruses in The Gambia remains poorly characterized. This study addresses this gap by investigating mosquito species composition and arbovirus prevalence across multiple habitats to generate baseline data on vector diversity and viral circulation. From December 2021 to October 2022, adult mosquitoes were sampled across sylvatic, agricultural, and human-inhabited sites in inland and coastal regions using CO_2-baited CDC light traps and BG-Pro traps. Specimens were identified morphologically, pooled by species, habitat, site, and season, and screened for arboviruses using viral isolation on Vero-76 cell lines. Viral identification was performed using RT-PCR with generic primers targeting alphaviruses, flaviviruses, and bunyaviruses, followed by Sanger sequencing. A total of 40,752 mosquitoes representing 52 species across 12 genera were collected. *Mansonia uniformis* predominated in sylvatic and agricultural habitats, while *Culex quinquefasciatus* dominated human-inhabited sites. Seasonality significantly influenced mosquito abundance, species richness, and Shannon diversity (LRT, $p < 0.001$), while habitat type was not significant. Arbovirus screening of 3,612 pools yielded five isolates: two Ngari virus, one Bunyamwera virus, and two Sindbis virus, all detected in October 2022 at Walikunda. Ngari virus was identified in *Anopheles gambiae* (MIR: 2.02%) and *M. uniformis* (MIR: 0.01%), Bunyamwera virus in *M. uniformis* (MIR: 0.01%), and Sindbis virus in *Culex neavei* (MIR: 0.01%) and *M. uniformis* (MIR: 1.19%). This study provides the first comprehensive assessment of mosquito diversity and arbovirus prevalence in The Gambia. Building on these findings, ongoing work investigates the vector competence of *Anopheles coluzzii* for Ngari virus to better understand vector-virus interactions.

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INFLUENCE OF INDOOR MICROCLIMATE ON *ANOPHELES FUNESTUS* RESTING PATTERNS IN SOUTH-EASTERN TANZANIA

Polius G. Pinda[1], Dickson S. Msaky[1], Gustav Mkandawile[1], Samwel Makayula[1], Antoine Cribellier[2], Constantianus J.M. Koenraadt[2], Florian T. Muijres[2], Fredros O. Okumu[3], Emmanuel W. Kaindoa[1]

[1]Ifakara Health Institute, Morogoro, United Republic of Tanzania, [2]Wageningen University, Wageningen, Netherlands, [3]University of Glasgow, Glasgow, United Kingdom

Indoor resting patterns of malaria vectors are changing, potentially driven by evolving housing designs (roofing materials and wall construction) and associated indoor thermal and humidity-steered microclimates. This study quantifies how microclimatic gradients shape the resting choices of *Anopheles funestus* in southeastern Tanzania, testing whether lowermost, midwall, and uppermost indoor zones create distinct thermal and humidity niches that drive mosquito site selection.Between November 2024 and January 2026, twelve sentinel houses were sampled three times a week. In each house, mosquitoes were collected from lowermost, wall-midsection and uppermost indoor zones using Prokopack aspirators. Mosquitoes were morphologically identified using standard taxonomic keys, and *Anopheles funestus* were included in the analysis. Indoor temperature and relative humidity were recorded at 30-minute intervals. Associations between microclimate and resting behavior were analyzed using mixed-effect models.Across 153 sampling days, 1,910 *An. funestus* were collected; 40% rested on lowermost surfaces. Uppermost sections had significantly fewer mosquitoes (aIRR = 0.40, CI: 0.31-0.51, $p < 0.001$). Metal-roofed and mud-walled houses had lower mosquito abundance than grass-thatched and brick-walled ($p < 0.001$). Indoor thermal microhabitats matched mosquito resting preference, for instance, uppermost surface, mosquito least preferred being the warmest compared to lowermost ($p < 0.001$; Cohen's $d = 0.72$ °C). Although, predicted mosquito abundance peaked jointly at temperatures of 32 °C and absolute humidity of 17 g/m^3, increased temperature fluctuation steered reduced abundance. Resting patterns of *An. funestus* were influenced by indoor microclimates, which are in turn shaped by house characteristics, particularly roof and wall materials. This highlights the importance of fine-scale microclimate dynamics on vector ecology and control strategies. For example, spatially targeted IRS, guided by species-specific resting behavior could improve intervention efficiency while reducing insecticide use and operational costs.

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INVASIVE *AEDES* (*AEDIMORPHUS*) *VITTATUS* IN MOMBASA CITY, KENYA: TRACING THE ORIGIN AND ITS POTENTIAL ROLE IN DISEASE TRANSMISSION

Joel Lutomiah[1], Mulwa Francis[1], James Kamami Mutisya[2], Solomon K. Langat[1], Chelangat Betty[1], Victor Okoth Anyango[3], Edith Chepkorir[1], Samson Limbaso Konongoi[4]

[1]Kenya Medical Research Institute (KEMRI), Nairobi, Kenya, [2]Kenya Medical Research Institute (KEMRI), NAIROBI, Kenya, [3]Lubbock, TX, United States, [4]Kenya Medical Research Institute, Nairobi, Kenya

Aede vittatus is a native species widely distributed in rural Kenya. Historically, this species was not reported in Mombasa City, Kenya. However, during a longitudinal entomological surveillance from 2014-2023 using standard entomological tools, n=4 *Ae. vittatus* were collected against *Ae. aegypti* (n=2065) in 2014. In January 2018, *Ae, vittatus* (n=1137) & *Ae. aegypti* (n=947) were collected. From June 2022 to July 2023, *Ae. vittatus* (n=1551; 51%) & *Ae. aegypti* (n=1509; 49%) were collected using BG-Sentinel (BGS) traps mostly at night, while human landing catches yielded n=1598 (42%) & n=2216 (58%) *Ae. vittatus* & *Ae. aegypti* respectively. *This* corresponded to 3.2 & 2.4 bites per person per hour for *Ae. aegypti* & *Ae. vittatus* respectively, which was not statistically significant (t=6.0081, df=1, p-value=0.105). *Virus screening using cell culture & qPCR yielded* chikungunya virus (CHIKV) from female *Ae. vittatus*. Analysis of mosquito bloodmeals by DNA amplification of 12S rRNA gene & sequencing revealed that *Ae. aegypti* preferentially fed on humans with human blood index (HBI) of n=33; 70%, cattle (n=6; 12.8%), cats (n=5; 10.6%), dogs (n=2; 4.3%), & mongoose (n=1; 2.1%), while *Ae. vittatus* (n=13; 100%) exclusively fed on humans, indicating high level of anthropophily. Metabarcoding of *Ae. vittaus* using cytochrome c oxidase I gene showed they only aligned with those from India, Sri Lanka & Nepal, & not those from rural Kenya. Alignment of the Mombasa *Ae. vittatus* with those from Asian countries suggest a recent introduction to Mombasa. Distribution of *Ae. vittatus* across Mombasa in numbers comparable to *Ae. aegypti*, similar human biting rates & total anthropophily suggest adaptation to urban environment. The presence of multiple vector species *Ae. vittatus* & *Ae. aegypti*, including *Culex quinquefasciatus,* from which CHIKV was isolated & confirmed to be competent, & nocturnal host seeking behavior enhances the risk of transmission, underscores the complex nature of dengue and chikungunya epidemiology & highlights the significance of integrated vector control strategies including use of insecticide treated nets at night.

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A COMPARISON OF INDOOR AND OUTDOOR MALARIA VECTORS IN A LONGITUDINAL COHORT STUDY IN WEBUYE, WESTERN KENYA.

Lucy Achieng Abel[1], Samuel Charo Kahindi[2], Daniel Onguru[3], Shehu Shagari Awandu[4], Mark Erickson Amunga[1], Millicent Cherono[1], Linda Auma Maraga[1], Dancan Wakoli[1], Evans Omollo[5], Erick Nalianya[5], Aggrey Wekesa[5], Steve Myer Taylor[6], Judith Nekesa Mangeni[7], Andrew Obala[8], Wendy P. O'Meara[6]

[1]Academic Model Providing access to Healthcare (AMPATH), Eldoret, Kenya, [2]School of Pure and Applied Sciences, Department of Biological Science, Pwani University, Kilifi, Kenya, Kilifi, Kenya, [3]School of Health Sciences, Jaramogi Oginga Odinga University of Science and Technology, Bondo, Kenya, [4]4.Department of Biological sciences, Jaramogi Oginga Odinga University of Science and Technology, Bondo, Kenya, [5]Duke Global Inc, Nairobi, Kenya, [6]Duke Global Health Institute, Duke University, Durham, NC, United States, [7]Moi UniveMoi University College of Health Sciences, School of Public Health, Eldoret, Kenya, [8]Moi University College of Health Science, School of Medicine, Eldoret, Kenya

Malaria remains a major public health challenge in Kenya, with ~75% of the population at risk. Its persistence is due to factors such as insecticide resistance, parasite drug resistance, weak healthcare system, socio-economic disparities, and climate change. The main malaria vectors in Webuye are *Anopheles gambiae s.s, An. arabiensis* and *An. funestus* that are known to be anthropophagic and endophagic. However, *An. arabiensis* has in recently been reported to shift towards exophagy and zoophillic feeding. These behaviors have been implicated in maintaining residual transmission. Despite scale-up of next-generation LLINs with PBO in the region, malaria burden remains high. Among the factors potentially sustaining transmission may be increased exophilic and exophagic vectors, thus reducing the efficacy of LLINs which protect humans indoors while sleeping. We compare indoor and outdoor malaria vector diversity, *P. falciparum* infection, and measured the blood meal sources for the outdoor vectors. For 12 months, collection of adult *Anopheles* mosquitoes by aspiration was done weekly in buildings with human sleeping spaces (indoor vectors), non-human dwellings and suspected mosquito resting areas (outdoor vectors). Mosquitoes were identified morphologically and laboratory analysis was conducted by PCR. Out of the 46,502 female mosquitoes collected,1,878 (4%) were anophelines. A total of 1,641 (87%) of the anophelines were collected indoors and 237 (13%) outdoors. Ten different *Anopheles* species were identified indoors, while seven were identified outdoors. *An. gambiae ss* was the most abundant vector both indoors and outdoors. A high proportion of the outdoor female *Anopheles* had bloodmeals from animals; only 3% fed on humans, and all were negative for sporozoites. The sporozoite rate for indoor collection was 4.8%. There was no evidence that outdoor resting mosquitoes are contributing to malaria transmission. However, the presence of several other species besides the traditional vectors poses a threat of cryptic species sustaining transmission and the ability to feed on animals implicated in sustaining vector populations.

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ECOLOGICAL DRIVERS OF WEST NILE VIRUS RISK AND VGSC MUTATIONS IN MOSQUITO VECTORS FROM ALBUQUERQUE, NEW MEXICO

Clinton O. Onyango[1], Sharley A. Wasena[1], Dianne Peterson[1], Miriam A. Betchel[1], Jessica T. Martin[2], Jessie L. Smith[2], Nicholas D. Pederson[2], Mark A. DiMenna[2], Douglas Jay Perkins[1], Ivy Hurwitz[1]

[1]Center for Global Health, University of New Mexico, Albuquerque, NM, United States, [2]City of Albuquerque, Environmental Health Department, Albuquerque, NM, United States

Mosquito-borne virus risk in arid urban environments is shaped by ecological factors and mosquito population dynamics. We analyzed long-term mosquito surveillance data from Albuquerque (2011-2024) and screened collections (2023-2025) for arboviruses and insecticide resistance markers. Surveillance at 23 sites along the Rio Grande valley yielded 98,108 *Culex quinquefasciatus* and 61,856 *Cx. tarsalis*, revealing consistent species-specific patterns. Generalized additive models showed that spatial variation among trapping sites explained most of the variation in mosquito abundance (81.6%; EDF=21.57; χ^2=1560.50). Temperature was the strongest weather driver (6.94%; EDF=8.29; χ^2=132.73), while combined effects of precipitation, temperature, and dew point (4.30%; EDF=15.04; χ^2=82.25) indicate abundance is driven by interacting weather conditions; precipitation alone was not significant. Female *Culex* collected during 2023-2025 were screened via duplex RT-qPCR for arboviruses. WNV infection rates were higher in *Cx. quinquefasciatus* (annual MLE: 16.3-22.3 per 1,000 mosquitoes) than *Cx. tarsalis* (6.0-13.2). Vector index peaks preceded statewide human WNV cases by ~2 weeks, supporting its use as an early transmission risk indicator. SLEV circulation was low, with one positive pool in 2023 and two in 2025. Genotyping of the voltage-gated sodium channel (VGSC) gene revealed widespread knockdown resistance (kdr) mutations. *Cx. quinquefasciatus* from pyrethroid-treated (79%) and untreated (90%) sites showed near-fixation of the L1014 kdr allele. *Aedes aegypti* collected during 2022-2024 exhibited high frequencies of resistance-associated mutations (V410L, V1016I, F1534C; ≥0.81), with triple-homozygous genotypes in 64-77% of individuals tested, indicating entrenched multi-locus kdr mutations. These findings demonstrate that WNV transmission risk in Albuquerque is shaped by spatial and weather-driven variation in *Culex* vectors. High frequencies of VGSC mutations in both *Culex* and *Aedes aegypti* indicate widespread target-site mutations associated with pyrethroid resistance in local vector populations.

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MOSQUITO-TRACKER: AN AI-DRIVEN BIOACOUSTICS AND CLIMATE MONITORING SYSTEM FOR MOSQUITO SURVEILLANCE

Nutifafa Yao Agbenor-Efunam, Akyana Britwum, Michael Kweku Edem Donkor, Rose-Mary Owusuaa Mensah Gyening, Alice Bagyiereyele Lakyiere, Edmund Ilimoan Yamba, Kingsley Badu

Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana

Mosquito-borne diseases remain a major global health threat, highlighting the need for effective surveillance tools for species identification and activity monitoring. We present Mosquito-Tracker, an IoT-enabled, AI-driven bioacoustic and climate monitoring system for real-time mosquito surveillance. The device uses a low-cost hardware suite including a microcontroller, GSM/GPS module, microphone, and temperature-humidity sensors. It records mosquito wingbeat sounds and environmental data from targeted locations, tagging each recording with GPS coordinates. Data are stored locally and can be transmitted to a cloud platform via Wi-Fi. Mosquito classification is performed using a custom convolutional neural network trained on mosquito wingbeat spectrograms to predict genus, sex, and age group, as well as a "not-mosquito" class, achieving 92% classification accuracy. Preliminary Grad-CAM analysis indicated that the network focuses on characteristic wingbeat frequencies for its predictions. By combining wingbeat acoustics with environmental data, Mosquito-Tracker enables precise surveillance and supports correlation of mosquito activity with local environmental conditions. The environmental data collected could also support future predictive models of mosquito abundance at specific locations. This approach supports early outbreak detection, enhances predictive models of mosquito-borne disease risk, and informs targeted vector control strategies.

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AI-ASSISTED VECTOR IDENTIFICATION FOR GLOBAL DEPLOYED US MILITARY UNITS

Margaret Glancey, Hannah Markle, Roy Faiman, Thomas Jenkins, Vasiliki Ikonomidou, Tristan Ford

VecTech, Baltimore, MD, United States

Rapid and accurate identification of disease vectors is critical for force health protection in military deployment settings, yet forward-deployed

preventive medicine technicians are tasked with surveillance responsibilities alongside numerous competing duties. The IDX device is an automated morphological identification system designed to identify medically important mosquito and tick species at the point of collection, without requiring entomological expertise. For the past several years, IDX has been deployed across five US military sites in the United States, Republic of Korea, Japan, and Kenya to further expand global species coverage in these regions. Recently, IDX has been used during field training operations to evaluate operational utility. Preliminary comparative data indicate that IDX outperformed identification accuracy and speed achieved by preventive medicine technicians using morphological keys in field conditions. Current species coverage encompasses key vectors across Africa and Asia, including *Aedes aegypti, Ae. albopictus, Anopheles stephensi, An. dirus, An. sinensis, Culex tritaeniorhynchus, Cu. pipiens s.l., and Mansonia uniformis,* among others. Comparing algorithm results to user contributed identifications (truth), the system achieved 99% precision and 83% recall for *An. gambiae sl* and 94% precision, 46% recall for *An. funestus sl* in African deployment settings. A May 2026 update will further expand malaria vector coverage with the addition of *An. longirostris*. These findings support IDX as a scalable, operationally practical tool for real-time vector surveillance in resource-constrained and high-operational-tempo environments, with implications for both military force protection and broader global health surveillance programs.

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COST COMPARISON OF COMMUNITY-BASED VECTOR SURVEILLANCE USING VECTORCAM: AN AI-ENABLED MOSQUITO IDENTIFICATION TOOL, VERSUS ROUTINE ENTOMOLOGICAL SURVEILLANCE IN UGANDA

Marina Rincon Torroella[1], Shreya Raman[1], Bryan Patenaude[1], Sunny Patel[1], Aryaman Shodhan[1], Soumyadipta Acharya[1], Winnie Kansiime[2], Jimmy Opigo[3], Peter Waiswa[4], Catherine Maiteki-Sebuguzi[3]

[1]Johns Hopkins University, Baltimore, MD, United States, [2]Makerere Univeristy, Kampala, Uganda, [3]Ministry of Health, Kampala, Uganda, [4]Makerere University, Kampala, Uganda

National malaria control programs depend on timely and reliable vector surveillance data to guide interventions. Current systems rely on analog data collection and microscopy-based morphological identification of mosquitoes by entomologists, a process constrained by limited experts, high specimen volumes, and specimen transport from rural collection sites.VectorCam is a smartphone application that uses artificial intelligence to classify mosquito species, sex, and abdominal status within seconds, enabling automated workflows and the task-shifting of some entomologist responsibilities to community health workers. This study evaluates the unit cost of integrating VectorCam into routine surveillance in Uganda. Data were extracted from a 12-month pragmatic parallel randomized trial conducted in two districts in Uganda (2023-2024). The analysis included costs directly associated with field activities: personnel, transport, and supplies. Three implementation models were assessed: (1) full task-shifting to Village Health Teams without Vector Control Officer involvement; (2) task-shifting with quarterly supervision; and (3) task-shifting with monthly supervision aligned with routine surveillance. A per-device and sensitivity analysis explored alternative deployment scenarios. The annual cost of routine surveillance (control arm) per district was USD10,615. In comparison, VectorCam reduced costs to USD 8,852 in Model 1 (16.6% savings), USD 9,200 in Model 2 (13.3 savings), and USD 9,896 in Model 3 (6.8 % savings). Cost reductions were driven by decreased personnel and transport, partially offset by technology-related supply costs. Each device cost USD 144.51 annually and replaces USD 438.29 in surveillance functions, resulting in net savings of USD 293.78 per device. Savings increased in scenarios with existing digital infrastructure or individual device use of VectorCam. Digitalizing vector surveillance with VectorCam offers a cost-saving and scalable approach for low- and middle-income countries by shifting resources from labor-intensive microscopy to technology operable by community health workers.

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CHARACTERISTICS OF HUMAN, PET AND DEER CONTACT CAPTURED ON SOCIAL MEDIA AND IMPLICATIONS FOR ZOONOTIC DISEASE TRANSMISSION, 2015-2025

Amira Roess, Emma Von Hoene, **Bridget Karably**, Ethan Hopson, Timur Ozkose, Tugce Gundogdu, Feyza Ozeren, Janay Hopgood, Zara Ahmed, Taylor Anderson

George Mason University, Fairfax, VA, United States

With increasing urbanization, human contact with deer is becoming more common. Monitoring emerging trends of such contact can identify behaviors that increase the risk of zoonotic disease emergence and transmission. Social media data can be used to learn about emerging trends in human and wildlife contact, yet this is not done and there are few systematic approaches to study this. We modified a systematic literature review approach to address this gap. The resulting protocol was finalized after three iterative pilot phases. Using the protocol, we identified videos posted from 2015-2025 on Facebook, TikTok, and Instagram that met the inclusion criteria (n=1332). Contact type (main outcome) and independent variable data (demographics, PPE use, pet presence, and metadata) were extracted. Frequencies were generated and a multivariable logistic regression assessed associations between contact type and select independent variables. TikTok videos have the most likes and longest durations. Most were recorded in spring and summer, during daytime. By state, Montana, Wyoming, Colorado, Missouri and Kentucky have the highest rate of geotagged videos per 100k population. Videos from the Midwest are more likely to capture feeding compared to other regions (p-val<0.0001). The demographic distribution of the person posting resembles that of the general U.S. population. Pets are present in 22.5% of videos. The most common contact types are observing with no direct physical contact (51.5%), touching/scratching/petting (38.9%), hand-feeding (12.2%), and rescuing (9.8%). People are significantly more likely to initiate contact than deer or pets are (p-val<0.0001). Only 2.9% of posters use PPE. This protocol is an important contribution to researchers interested in conducting systematic research using social media to better understand human and animal contact. The observed behaviors raise public health concerns about zoonotic disease transmission, animal welfare concerns, and injury risks.

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EVIDENCE-BASED FRAMEWORK FOR PRIORITIZING RIFT VALLEY FEVER VACCINATION IN ENDEMIC SETTINGS

Rosemarie Elizabeth Scharf[1], Woutrina Smith[1], Brian H. Bird[1], Grace Mwangoka[2], Mwokozi Mwanzalila[2], Ana Pérola Brandão[1], Robert Sumaye[2], Cheryl Meehan[1], Sonja Montanus[1], DDvax Consortium[1]

[1]University of California Davis School of Veterinary Medicine, One Health Institute, Davis, CA, United States, [2]Ifakara Health Institute, Bagamoyo, United Republic of Tanzania

Rift Valley fever (RVF) is a zoonotic disease causing significant public health and economic burden across Africa and the Arabian Peninsula, with transmission driven by livestock-vector-human interfaces. Despite vaccination being a key intervention for outbreak prevention and control in both human and animal populations, evidence-based guidance to prioritize high-risk populations is limited. We developed a quantitative prioritization framework integrating three layers: (1) geographic risk based on seroprevalence, (2) behavioral exposure reflecting high-risk activities (e.g., livestock handling, animal births, mosquito exposure), and (3) transmission amplification and intervention feasibility, including mobility, livestock contact patterns, healthcare access, and RVF awareness and vaccine acceptability. Behavioral and feasibility components were informed by knowledge, attitudes, and practices (KAP) surveys conducted in coastal and south-central Tanzania, capturing vaccine awareness, willingness to vaccinate, and socio-cultural determinants of uptake. These data highlighted low-to-moderate awareness of RVF, moderate willingness to accept a potential RVF vaccine, and structural and socio-cultural barriers to uptake. The three

layers were integrated into a Composite Priority Index (CPI) to rank wards for vaccination prioritization. High-risk populations were identified where livestock exposure and transmission potential converge, particularly in settings with low awareness and limited access to prevention resources, supporting integrated human-livestock vaccination strategies with community engagement. This One Health framework builds on spatial risk mapping approaches used for vector-borne diseases such as dengue and aligns with targeted vaccination strategies used in Ebola outbreaks. The CPI operationalizes epidemiologic data into actionable vaccination prioritization and is adaptable to other zoonotic diseases, demonstrating a practical One Health approach to outbreak prevention and control. This work is timely, as it aligns with and supports upcoming RVF vaccine trials.

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ONE HEALTH SURVEILLANCE APPROACH ILLUMINATED SILENT SLEEPING SICKNESS TRANSMISSION HOTSPOTS IN TSETSE INFESTED HAMLETS OF OYO STATE, NIGERIA

Rolayo Toyin Emmanuel[1], Kelly Zongo[2], Olaleye O. Olusola[3], Temitope Popoola[3], Melemi R. Mazadu[1], Emmanuel Enoh[1]

[1]Nigerian Institute for Trypanosomiasis and Onchocerciasis Research, Kaduna, Nige, Kaduna, Nigeria, [2]The END Fund, New York, NY, United States, [3]Nigerian Institute for Trypanosomiasis and Onchocerciasis Research, Kaduna, Nige, Ibadan, Nigeria

Approximately 55 million individuals in sub-Saharan Africa face the peril of contracting sleeping sickness (SS). Its occurrence is driven by an epidemiologic triad comprising *Trypanosoma brucei gambiense*, tsetse flies, humans, animals and a propitious environment. Despite advancements in diagnostics, the actual status of sleeping sickness (SS) in Nigeria remains elusive. In our quest for clarity, we embraced a holistic approach: One Health surveillance comprising human and animal population surveys, entomological and ecological assessments to illuminate SS transmission hotspots and risk factors in sparsely-populated remote rural hamlets. We collected blood samples from 72 consenting humans and 145 animals. Furthermore, tsetse flies were captured using odour-baited traps, species diversity and spatial distribution were assessed. Captured tsetse, human and animal blood samples were screened for *T. b. gambiense* infection using TgsGP-specific colorimetric loop-mediated amplification. Tsetse bloodmeals were analyzed. Captured tsetse were *Glossina palpalis palpalis* with 16.90% *T. b. gambiense* infection rate. Humans were preferred bloodmeal source (41.67%, p= 0.010). Alaho has the highest tsetse density 10.33 flies/trap/day, highest human-tsetse contact and transmission risk index of 80787.31. We found that anthropogenic activities and ecological conditions impacted tsetse density (p< 0.0001). Prevalence of *T. b. gambiense* among humans and domestic animals was 40.28% and 40.69% respectively. Illiteracy (p= 0.01) and defecation in forests (p= 0.0004) were major determinants of SS occurrence. We identified thirteen SS transmission hotspots. Correlation exists between humans, animals, and tsetse infections (p< 0.0001), depicting One Health implication. Silent transmission of asymptomatic SS poses a significant unaddressed threat, warranting intensified sensitization and surveillance. Animals serve as reservoir host aiding persistence of SS in the hamlets. Urgent One Health and WASH-guided strategic control approaches are imperative to prevent SS epidemic in the hamlets and devastating resurgence in Nigeria.

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INTEGRATING RODENT SPACE USE AND HUMAN MOVEMENTS TO MODEL SPATIAL PATTERNS OF ZOONOTIC EXPOSURE ACCROS LAND-USE GRADIENTS IN WESTERN UGANDA

Fanohinjanaharinirina Rasoamalala[1], Emilia Johnson[2], David Kiragu[2], Christabel Achieng[3], Gladys Atim[4], Barbara Han[5], Christina Faust[6], James Hassell[1]

[1]Simthsonian Institute, Washihgton, WA, United States, [2]Glasgow University, Glasgow, United Kingdom, [3]International Livestock Research Institute, Nairobi, Kenya, [4]New Haven, CT, United States, [5]Cary Institute of Ecosystem Studies, Millbrook, NY, United States, [6]University of Glasgow, Glasgow, United Kingdom

The risk of zoonotic diseases stems from complex interactions between wildlife reservoirs, environmental conditions and human behaviour. Indeed, the mechanisms linking land use to the viral risk transmitted by rodents, as well as human exposure, remain poorly quantified at fine spatial scales. This study aims to quantify the spatial distribution of rodents and integrate this with human movements in order to estimate the spatial patterns of exposure to zoonoses across land-use gradients in western Uganda. Fieldwork was carried out at six sites representing gradients in land-use systems, such as agricultural land, forest edges and intact forests. Small mammals were sampled using trap grids; individuals were captured, fitted with Pit Tags and released. The movements and activity of rodents were estimated and quantified using a combination of rhodamine B bait marking, abundance using tiles, and RFID (radio-frequency identification) tags as long-term tracking devices. Human movements were assessed using GPS tracking of one representative per household over seven days, supplemented by demographic and behavioural surveys. Generalised linear mixed model and a hierarchical Bayesian spatial modelling framework were used to quantify variation in rodent space use, human movement, and interaction across the land-use types. A total, 953 individuals representing 11 species were captured across wet and dry seasons, with a marked predominance of the genus *Mastomys*. Among these, 151 individuals tested positive for Rhodamine B. The maximum observed movement distance reached 156 m from the marking points, with variations depending on the land-use gradient and rodent species. Rodent activity and their presence in dwellings were higher in forest edge and agricultural areas compared to areas of intact forest. Rodent use of space constitutes a key mechanistic link between environmental changes and human exposure to zoonotic pathogens. This framework promotes a better understanding of the transmission dynamics of zoonoses and can inform targeted surveillance and intervention strategies in heterogeneous landscapes.

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COMPREHENSIVE ECO-EPIDEMIOLOGICAL SURVEILLANCE AND COMMUNITY-BASED INTERVENTION FOR LEISHMANIASIS IN A NORTHWESTERN COLOMBIAN DEPARTMENT: A ONE HEALTH APPROACH

Yeiner Miguel Espitia Delgado[1], Maria Fernanda Yasnot[2]

[1]Universidad de Córdoba, Montería, Colombia, [2]Monteria, Colombia

Leishmaniasis is a complex public health challenge in Colombia requiring integrated vector surveillance, reservoir management, active case finding, and community education. We present a multidisciplinary intervention in endemic municipalities of a northwestern Colombian department to strengthen disease prevention and local public health capacities. A multi-component strategy was deployed. Entomological surveillance utilized CDC light traps in high-risk areas. Domestic canine reservoirs were screened using rK39 rapid tests. Active human case finding by community health brigades utilized rK39 testing, molecular diagnostics, and parasite isolation. Concurrently, educational campaigns were conducted in schools and communities. Capacity building involved 80-hour hybrid training courses in clinical management and Primary Health Care for local health personnel. Entomological surveys yielded 337 phlebotomine sand flies, predominantly *Lutzomyia gomezi* (n=124), *L. evansi* (n=94), and *L. panamensis* (n=91). Serological screening of 156 canines identified 8 positive cases, 6 clustered within the indigenous community of El Delirio in San Andrés de Sotavento. Active screening of 304 individuals detected 3 human cases. Educational interventions reached 605 individuals in schools and 502 community members. Additionally, 102 healthcare workers were trained (52 clinical, 50 primary care). This integrated approach identified active transmission microfoci while strengthening local diagnostic, clinical, and community capacities. Combining robust eco-epidemiological surveillance with targeted educational interventions provides an effective, sustainable model for leishmaniasis control in vulnerable regions.

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A ONE HEALTH GEOSPATIAL FRAMEWORK FOR *LEPTOSPIRA* SURVEILLANCE: INTEGRATING UPLAND ENVIRONMENTAL PERSISTENCE AND HUMAN SEROPREVALENCE ON O'AHU, HAWAI'I

Vivek R. Nerurkar, Rodson Zorilla, Colby Macapagal, Duy LN Tran, Cecilia M. Shikuma

University of Hawaii School of Medicine, Honolulu, HI, United States

Leptospira transmission within Hawai'i's ecosystems is shaped by a complex One Health nexus, where pathogen stability is governed by the mountain-to-sea hydrologic continuum. This study applies a multivariate geospatial workflow to identify ecological drivers of *Leptospira* in soil and water reservoirs, from forested headwaters to urbanized coasts, and to assess the immunologic impact on human populations across the hydrologic continuum of O'ahu. A comprehensive geospatial model was developed in ArcGIS Pro, synthesizing field-observed data with edaphic datasets (USDA SSURGO), USGS topography, and 2020 Decennial Census variables. The sampling strategy targeted the forest-urban-stream interface, collecting paired specimens from lotic freshwater systems and adjacent saturated riparian soils, as well as high-contact recreational and flood-prone areas. Pathogen density was quantitated via qPCR (Genomic Equivalents, GE), while a concurrent cross-sectional serosurvey characterized the corresponding human immunologic footprint. *Leptospira* DNA was detected in 44% (8/17) of aqueous sites and 39% (7/17) of terrestrial sites, with the highest biological burdens observed in forested riparian corridors (p=0.03). Regression analysis revealed distinct contamination dynamics: waterborne GE showed a positive correlation with urban population density (r=0.44, p=0.037), whereas soil reservoirs remained statistically independent of anthropogenic development (p=0.954). These findings suggest that peririverside soils serve as stable ecological niches, likely maintained by sylvatic reservoirs such as feral swine. Multivariate linear regression identified soil pH and population density as the primary predictive covariates (r = 0.45). Contemporary human seroprevalence (2.7%) did not differ significantly (p=0.28) from the 3.8% reported in 1943, indicating stable environmental pressure despite eight decades of rapid urbanization. These findings demonstrate that streams and their associated soil interfaces are primary drivers of *Leptospira* endemicity in Hawai'i.

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KNOWLEDGE PATHWAYS, TRUST, AND ZOONOTIC RISK AMONG ARAB BEDOUIN LIVESTOCK OWNERS: APPLICATION OF THE RELATIONAL ONE HEALTH FRAMEWORK

Haneen Shibli[1], Julianne Meisner[1], Anat Rosenthal[2]

[1]University of Washington, Seattle, WA, United States, [2]Ben Gurion University of the Negev, Beersheba, Israel

Brucellosis remains a significant zoonotic threat in settings where human livelihoods, ecosystems, and animal health are deeply intertwined. The Arab Bedouin minority in Israel face a disproportionate burden due to structural marginalization, limited access to veterinary and healthcare services, and rapid urbanization, which shape human-animal-environment interactions and govern how livestock health knowledge is interpreted and applied. Guided by the Relational One Health framework, this study used qualitative methods to investigate how Bedouin households interpret and act on information about brucellosis, and how knowledge pathways and trust influence practice. Between May 2025 and January 2026, we conducted 29 key informant interviews, 30 oral histories, 12 transect walks, and 12 focus group discussions across 11 communities. Interviews were conducted in Arabic, transcribed verbatim, and analyzed thematically. Participants described a range of understandings of livestock health, from accurate knowledge to partial or inconsistent interpretations. Participants expressed confidence in protective practices including boiling milk, while others highlighted gaps in awareness, particularly in remote areas. Knowledge was shaped by veterinarians, family experience, community norms, and traditions. Trust was a key factor in engagement with One Health systems, with experiences ranging from skepticism toward governmental veterinary services to selective reliance on community-based or private providers. Limited availability, inconsistent followup, high costs, and perceived unequal enforcement contributed to feelings of neglect and influenced herd health decisions. Findings demonstrate that Bedouin households draw on layered and uneven knowledge systems when navigating brucellosis risk. The findings highlight the value of a Relational One Health approach that integrates communityrooted expertise while strengthening access to reliable information. Supporting these connections may help reduce brucellosis transmission and support healthier human-animal-environment relationships worldwide.

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INTEGRATING ENVIRONMENTAL SURVEILLANCE AND COMMUNITY EDUCATION FOR SCHISTOSOMIASIS CONTROL: A ONE HEALTH APPROACH IN OGUN STATE, NIGERIA

William Cormack McCarthy[1], Olubukola Adelakun[2], Cristina Salas[3], Richa John[1], Cynthia Umunnakwe[4], Julianne Wu[5], Bhargavi Limbachiya[5], Caroline Crain[6], Simon Lawson Birk[7], Thomas Copeland[8], Daniel Amao[9], Rakesh Kathiresan[1], Andy Chamberlin[10], Ifeoluwa George[11], Uwemedimo Friday Ekpo[11], Manu Prakash[12]

[1]University of Texas Health Science Center at Houston, McGovern Medical School, Houston, TX, United States, [2]Oyo State College of Agriculture and Technology, Ibadan, Nigeria, [3]University of Texas Medical Branch John Sealy School of Medicine, Galveston, TX, United States, [4]Federal University of Agriculture, Odeda, Nigeria, [5]Harvard, Cambridge, MA, United States, [6]UTHealth McGovern Medical School, Houston, TX, United States, [7]Houston, TX, United States, [8]Health In Your Hands Diagnostics, 501 (C)(3), Houston, TX, United States, [9]Health In Your Hands Diagnostics, 501 (C)(3), Houston, TX, United States, Lagos, TX, United States, [10]Stanford Woods Institute, Stanford, CA, United States, [11]Federal University of Agriculture Abeokuta, Abeokuta, Nigeria, [12]Stanford University, Stanford, CA, United States

Sustainable schistosomiasis control requires community-led interventions addressing environmental transmission risk and health literacy. We present a One Health approach developed with endemic communities along the Oyan Reservoir, Ogun State, Nigeria. Drone-based vegetation mapping across four communities (n=134 schoolchildren, prevalence 38.1%) identified aquatic vegetation coverage as strongly correlated with community-level schistosomiasis prevalence (Pearson r=0.951, submerged vegetation r=0.968, Spearman ρ=1.000). Water contact sites were community-identified, complementing remote sensing with local knowledge. Quadrat sampling identified the predominant submerged vegetation as *Ceratophyllum demersum*, an invasive macrophyte, with significantly higher *Bulinus* snail densities in submerged versus emergent zones, establishing a targetable transmission habitat. Satellite imagery will extend risk mapping across 18 communities. Farmers, fishermen, and community leaders will be engaged through semi-structured interviews on community-led *C. demersum* removal, with harvested plants dried for cattle feed or composted as fertilizer, creating economic incentives for sustained snail control. Surveys revealed a striking literacy gap: while 91% of schoolchildren had heard of schistosomiasis and 75% had been previously treated, fewer than 34% could name a single complication. MicroSafari: Hidden Worlds, co-designed with Nigerian children's book artist Henry Ezeokeke and local schoolteachers, uses the Foldscope, an accessible $2 paper microscope, to bridge this gap. Biological specimens embedded in reusable magnetic puzzle pieces serve as interactive microscope slides, rearranged on a landscape board to trace the parasite's life cycle through human, cattle, and snail hosts across local vegetation habitats. Children identifying symptoms are referred to community health extension workers for praziquantel treatment in collaboration with the Ogun State Ministry of Health. Impact will be assessed through Knowledge, Attitudes, and Practices surveys and CHEW-recorded self-referral rates.

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PHENOTYPIC AND GENOTYPIC IDENTIFICATION OF *PROTEUS MIRABILIS* ISOLATED FROM CULTURED CATFISH AND TILAPIA AND IMPLEMENTATION OF HAZARD ANALYSIS AND CRITICAL CONTROL POINT-BASED BIOSECURITY MEASURES FOR THE CONTROL OF FISH-BORNE PATHOGEN IN AQUACULTURE SYSTEMS.

Enow Canicia Efundem[1], Rhoda Nsen Bughe[1], Ellison R. Amy[2], Wilfred Fon Mbacham[1]

[1]University of Yaounde 1,Cameroon, Yaounde, Cameroon, [2]Bangor University, UK, wales, United Kingdom

Fish-borne bacterial infections represent a significant threat to food safety and aquaculture productivity in Sub-Saharan Africa that is rapidly expanding. Increasing reports of zoonotic transmission of multidrug-resistant *Proteus mirabilis* highlight the urgency of improved surveillance and control strategies. This study investigated the occurrence, virulence and antibiotic resistance profile of *P. mirabilis* in cultured *Oreochromis niloticus* (tilapia) and *Clarias gariepinus* (catfish), HACCP-based biosecurity measures is proposed in aquaculture systems. A total of 150 live fish samples (75 tilapia and 75 catfish) were collected from ponds across three zones in Yaoundé. Isolation and identification were conducted using standard microbiological, biochemical tests, and confirmation with VITEK® 2 Compact system. Molecular characterization was performed using PCR targeting *ureC* and *zapA* virulence genes. Antibiotic susceptibility was assessed using the Kirby-Bauer disk diffusion method. Thirty-seven *P. mirabilis* isolates were identified, comprising 14 tilapia and 23 catfish, with 100% confirmation by PCR. Virulence profiling: high prevalence of *ureC* (100%) and *zapA* (94.7%), indicating strong pathogenic potential. Antimicrobial testing revealed multidrug resistance, against β-lactams and tetracyclines, susceptibility remained high for aminoglycosides and fluoroquinolones. All isolates were susceptible to carbapenems, moderate susceptibility for cefepime and intermediate resistance to cefotaxime and ceftriaxone. HACCP analysis identified critical control points in pond management: water quality, feed handling, fish health monitoring, stocking density, equipment sanitation and post-harvest practices. Integrating PCR-based diagnostics into routine monitoring is recommended for early detection and control.This study highlights the public health risks posed by multidrug-resistant *P. mirabilis* and demonstrates that integrating HACCP-based biosecurity with molecular diagnostics can enhance food safety, limit antimicrobial resistance and promote sustainable aquaculture.

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EFFECTS OF INTERMITTENT PREVENTIVE TREATMENT IN CHILDHOOD WITH MONTHLY DIHYDROARTEMISININ-PIPERAQUINE ON INFANT GROWTH OUTCOMES

Anna T. Nguyen[1], Michelle E. Roh[2], Abel Kakuru[3], Florian Bach[1], Jimmy Kizza[4], Maato Zedi[5], Miriam Aguti[5], Joaniter Nankabirwa[6], Moses R. Kamya[7], Grant Dorsey[8], Prasanna Jagannathan[9], Jade Benjamin-Chung[9]

[1]Stanford University, Palo Alto, CA, United States, [2]University of California, San Francisco, Portland, OR, United States, [3]Infectious Diseases Research Collaboration, Tororo, Uganda, [4]Infectious Diseases reseaech collaboration, BUSIA, Uganda, [5]Infectious Diseases Research Collaboration, Kampala, Uganda, [6]Makerere Univ Kampala, Kampala, Uganda, [7]Makerere University, Kampala, Uganda, [8]Univ of California San Francisco, San Francisco, CA, United States, [9]Stanford University, Stanford, CA, United States

Intermittent preventive treatment (IPT) in childhood with dihydroartemisinin-piperaquine (IPTc-DP) nearly eliminates the risk of malaria, but it is unclear how this affects long term infant health. Moreover, there is growing concern that IPT with DP during pregnancy may contribute to fetal growth restriction, but no studies have investigated DP's impact in infancy. Here, we assessed the effect of IPTc-DP on infant growth in a randomized controlled trial conducted in Busia, Uganda. Children were randomized to one of three groups: monthly IPTc-DP from 2-12 months, monthly IPTc-DP from 2-24 months, or placebo. Height and weight were measured during monthly routine visits. We estimated prevalence ratios (PR) for stunting (length-for-age z-score < -2) and wasting (weight-for-length z-score < -2) using log-binomial models with robust standard errors, adjusting for baseline maternal, birth, and household factors to account for chance imbalances. In the first three years of life, children on IPTc-DP were more likely to experience a wasting event compared to placebo (30.3% vs 24.5%; PR 1.21, 95% CI 0.92, 1.58), though confidence intervals included the null. Increased wasting prevalence among children receiving IPTc-DP vs. placebo began at 10 months and persisted through 36 months, with the largest differences from 16-18 months: wasting prevalence peaked at 16.5% among children who stopped DP after 12 months, compared to 12.7% (PR 1.43, 95% CI 0.93, 2.20) among children who continued DP through 24 months and 9.9% (PR 1.81, 95% CI 1.14, 2.87) in placebo. We observed similar, but weaker, effects of IPTc-DP on continuous measures of weight-for-length z-scores. There was slightly higher stunting prevalence among children receiving IPTc-DP vs placebo between 10-12 months (19.9% vs 15.8%; PR 1.38, 95% CI 1.0, 1.9), but we observed similar stunting risks by treatment arm at all other ages. While IPTc-DP is extremely effective at preventing malaria, it may increase the risk of acute malnutrition during an important period for child growth. Future work is needed to assess whether these transient effects on malnutrition influence other developmental outcomes.

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MALARIA CHEMOPREVENTION IN SCHOOL-AGE CHILDREN: A RANDOMIZED CONTROLLED TRIAL COMPARING DIHYDROARTEMISININ-PIPERAQUINE AND SULFADOXINE-PYRIMETHAMINE PLUS AMODIAQUINE TO CONTROL IN MALAWI

Wongani John Nyangulu[1], Enalla Mzembe[2], Ulemu Kawelama[2], Alfred Martin Matengeni[2], Fred Chinzimu[3], Francis Chiumia[4], Felix Khuluza[4], James Chirombo[5], Miriam Laufer[6], Don Pascal Mathanga[4], Lauren Cohee[1]

[1]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [2]Malaria Alert Centre, Blantyre, Malawi, [3]Blantyre Malaria Project, Blantyre, Malawi, [4]Kamuzu University of Health Sciences, Blantyre, Malawi, [5]Malawi Liverpool Wellcome Trust Clinical Research Programme, Blantyre City, BL, Malawi, [6]Univ of Maryland, Baltimore, MD, United States

School-age children experience a high but underappreciated burden of malaria infection and morbidity. The WHO recommends intermittent preventive treatment for malaria in school-age children (IPTsc), but adoption remains low. Dihydroartemisinin-Piperaquine (DP) has emerged as the drug of choice for IPTsc but due to concerns over artemisinin resistance and restrictions to use of first line antimalarial treatments for chemoprevention, a non-artemisinin alternative is required. We conducted a randomized controlled trial among primary school children in rural Blantyre, Malawi who were allocated to the following arms, 1) IPTsc with DP, 2) IPTsc with Sulfadoxine-Pyrimethamine plus Amodiaquine (SPAQ), and 3) Control (no treatment). During four six-weekly follow up visits, participants in the treatment arms received full courses of study drugs. Blood specimens for *Plasmodium falciparum* PCR and haemoglobin were collected at the first intervention visit and at the outcome visit done concurrently with the fourth intervention visit. The primary outcome was *Pf* as detected by PCR. Secondary outcomes were anaemia, clinical malaria incidence, haemoglobin and absenteeism from school. We enrolled 788 children and obtained primary outcome results for 82% (644) participants. Among the enrolled, mean age was 9.95 (SD 3.44), 52% were female, and 58% reported sleeping under a bed net. At the outcome, the prevalence of *Pf* as was 3.3% (7/204), 2.9% (6/201) and 12.1% (25/181) in the DP, SPAQ and Control arms respectively. Compared to control, both DP (adjusted OR [aOR]:0.24, 95% CI 0.09-0.55, p=0.001) and SPAQ (aOR 0.22, 95% CI 0.08-0.51, p=0.001) significantly reduced *Pf* prevalence. Both treatments reduced clinical malaria episodes (DP: aIRR 0.45, 95% CI 0.37-0.54, p<0.001; SPAQ: aIRR 0.38, 95% CI 0.30-0.47, p<0.001). SPAQ, only, increased haemoglobin (MD 0.23, 95% CI 0.015 - 0.451, p=0.036) but neither significantly reduced anaemia nor absenteeism. In Malawi and

settings with similar malaria transmission and resistance patterns, SPAQ is a suitable non-artemisinin alternative to DP for intermittent preventive treatment of malaria in school children.

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SUSTAINED REDUCTION OF MALARIA ASYMPTOMATIC PARASITAEMIA FOLLOWING MALARIA MASS DRUG ADMINISTRATION IN THE EASTERN REGION OF GHANA: A PILOT CLUSTER RANDOMISE TRIAL

Ndong Ignatius Cheng[1], Ndong H. Ndang[2], Chuo Ennestine Chu[3], Mustapha Dibbasey[1], Nuredine Mohammed[1], Collins Stephen Ahorlu[4], Alfred Amambua-Ngwa[1]

[1]MRC, Banjul, Gambia, [2]Universit of Buea, Buea, Cameroon, [3]NMIMR, Accra, Ghana, [4]Noguchi Memorial Institute for Medical Research, Accra, Ghana

Mass drug administration (MDA) is a strategy aimed at reducing the human parasite reservoir and interrupting malaria transmission. Evidence from cluster-randomized trials in West Africa remains limited. This study evaluates the sustained impact of MDA on *Plamodium falciparum* parasitaemia in endemic communities. This was conducted across 18 clusters (9 intervention, 9 control). The intervention arm received 6 rounds of bi-monthly MDA using dihydroartemisinin Piperaquin alongside standard care provided by community health volunteers from door-to-door, while the control arm received standard care only. Individual and cluster-level data were collected at baseline and endline, including age, sex, insecticide-treated net (ITN) usage, baseline malaria status, and rapid diagnostic test (RDT) results. Post-MDA, three follow-up surveys were conducted every 4-months alongside booster doses in the intervention arm, while the control arm simultaneously received 3 MDA rounds. Prevalence ratios (PR) and adjusted prevalence ratios (aPR) with 95% confidence intervals (CI) were calculated. Subgroup analyses were performed by age, sex, ITN use, and baseline malaria status. At endline, RDT positivity was 28.0% (236/852) in control clusters versus 1.5% (15/976) in intervention clusters (PR 0.06, 95% CI: 0.02-0.11; aPR 0.07, 95% CI: 0.02-0.10; $p < 0.001$). These reductions were consistent across demographic and baseline infection subgroups. Among children ≤15 years, the aPR was 0.48 (95% CI: 0.41-0.56). In the follow-up study, prevalence in the control arm dropped to 0.66% by July 2025 compared to 0.87% in the intervention arm. Furthermore, in February 2025, a prevalence of 8.95% was observed in the control arm, contrasting with 1.84% in the intervention arm. MDA significantly reduced malaria parasitemia across all demographic and baseline infection subgroups, underscoring its effectiveness as a population-level intervention in endemic areas. Follow-up results reveal sustained low levels of malaria positivity in the intervention arm, suggesting that integration with routine malaria control strategies may enhance elimination efforts.

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FIELD VALIDATION OF ASPIRATIONAL INSECTICIDE-TREATED NETS IN MALAWI: EVIDENCE OF IMPROVED DURABILITY AT HIGHER RESISTANCE-TO-DAMAGE SCORES

Matt Worges[1], Maurice Tauzie[2], Frank Mechan[3], Steve Poyer[4], Eleanore D. Sternberg[5], Christen Fornadel[6], Julie-Anne Akiko Tangena[3], John Gimnig[7], Themba Mzilahowa[8]

[1]Tropical Health, Tel Aviv, Israel, [2]Malaria Alert Centre, Blantyre, Malawi, [3]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [4]Tropical Health, Bristol, United Kingdom, [5]Tropical Health, Liverpool, United Kingdom, [6]IVCC, Liverpool, United Kingdom, [7]CDC, Atlanta, GA, United States, [8]Univ of Malawi, Blantyre, Malawi

Insecticide-treated nets (ITNs) are expected to provide three years of protection, yet field evidence shows that physical deterioration often leads to earlier failure. We conducted a longitudinal durability study in Balaka district, Malawi, to assess the field performance of ITNs with varying resistance-to-damage (RD) scores, including aspirational designs. Approximately 1,700 nets were distributed across seven sites, with net brands randomized to households within each site to promote comparable environmental and usage conditions across products. Three net categories were evaluated: standard nets (RD 39.5-65.8), a reinforced net (RD 61.0), and two aspirational nets from different manufacturers (RD 79.7 and 82.3). Nets were followed at six-month intervals and assessed for attrition and physical integrity, with failure defined as loss or non-serviceable condition. Cox proportional hazards models were used to estimate differences in survival between net types. Aspirational nets demonstrated substantially improved durability, with a 70% lower hazard of failure compared to standard and reinforced nets (HR 0.30, 95% CI: 0.22-0.41; $p < 0.0001$). Median survival increased from approximately 2.0 years for standard nets to 3.3 years for the reinforced net and 4.0 years for aspirational nets. These findings provide experimental field evidence that higher RD scores translate into meaningful gains in ITN durability and support the use of RD-based metrics to guide product selection and innovation.

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GPS-BASED COVERAGE METRICS FOR EVALUATING LARVAL SOURCE MANAGEMENT

Jordan Smith[1], David Galick[2], Duncan McFarland[1], Lucas Ondo[2], Liberato Motobe[2], Restituto Mba Nguema[2], Jeremias Nzamio[2], Wonder Philip Phiri[2], Guillermo A. Garcia[1], Carlos Alberto Guerra[3]

[1]MCD Global Health, Hallowell, ME, United States, [2]MCD Global Health, Malabo, Equatorial Guinea, [3]MCD Global Health, Quito, Ecuador

Larval source management (LSM) is a key complementary malaria control strategy, but accurately measuring operational coverage remains challenging. The Bioko Island Malaria Elimination Project has expanded LSM activities since 2023 in predominantly urban and peri-urban settings. Coverage has been assessed through supervisor recall across predefined 100 m × 100 m sectors, with a target of visiting each sector at least every three weeks. In 2026, GPS tracking was introduced to enable objective and granular measurement of spatial coverage and scouting effort. We conducted a preliminary observational analysis of LSM coverage. We quantified operational effort (field-days, and time and distance estimated from GPS data), developed and compared coverage metrics based on supervisor-reported sector visits, GPS-confirmed sector visits, and GPS-confirmed sub-sector (25 m × 25 m) visits. We assessed associations between scouting effort and larval habitat detection using a negative binomial regression, controlling for building density. Both supervisor-reported coverage and GPS-confirmed sector coverage were very high (99% vs 96%). However, only 51% of sectors achieved 100% sub-sector coverage according to GPS tracking. Search effort variables were the primary drivers of habitats detected. Intuitively, each additional sub-sector scouted and each additional 15 minutes of scouting were significantly associated with a 31% and 67% increase in habitats detected, respectively. Conversely, each additional kilometer traversed was significantly associated with a 58% decrease, probably confounding the time spent required at evaluating sites found. Our findings reveal that recall-based methods overestimate coverage but critically fail to capture intra-sector search coverage. GPS-derived metrics provide a more objective framework for evaluating coverage, linking effort to outcomes, improving monitoring and strengthening LSM effectiveness.

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SUBMICROSCOPIC MALARIA INFECTIONS AND INADEQUATE INTERMITTENT PREVENTIVE TREATMENT IN PREGNANCY WITH SULVADOXINE PYRIMETHAMINE COVERAGE AMONG PREGNANT WOMEN IN RURAL BURKINA FASO: A PROSPECTIVE COHORT STUDY

Yssimini Nadège Guillène TIBIRI[1], Issiaka Soulama[2], Dramane Zongo[1], Oumarou Ouédraogo[1], Salif Sombie[3], Ouédalo Nadège Millogo[1], Lucien Platon[4], Séni Nikiema[3], Wéta Fabiola Saoudia Diarra[1], Julien Nassandba Yannogo[1], Oumou Aicha Djamila Zeina Zoure[1], Raissa Pougsada Ily[1], Salam Sawadogo[1], Alioune Wade[4], Buze Chala[4], Lucas Thiebaut[4], Emmanuelle Caspar[4], Pierre-Emeric Strubel[4], Allan Muller[4], Patrice Piola[5], Cheikna Zongo[6], Didier Menard[4]

[1]Institut de Recherche en Science de la Santé (IRSS), Ouagadougou, Burkina Faso, [2]Institut de Recherche en Sciences de la Santé (IRSS), Ouagadougou, Burkina Faso, [3]Centre Nationale de Recherche et de Formation sur le Paludisme (CNRFP), Ouagadougou, Burkina Faso, [4]Malaria Genetics and Resistance Team (MEGATEAM), UR 3073 - Pathogens Host Arthropods Vectors Interactions (PHAVI) Unit, Université de Strasbourg, Strasbourg, France, [5]WorldWide Antimalarial Resistance Network (WWARN), Oxford, United Kingdom, [6]Laboratoire de Biochimie et Immunologie Appliquées (LABIA), Université Joseph KI- Zerbo, Ouagadougou, Burkina Faso

Malaria in pregnancy remains a major cause of maternal and neonatal morbidity in sub-Saharan Africa. Intermittent preventive treatment with sulfadoxine-pyrimethamine (IPTp-SP), delivered presumptively at antenatal care (ANC) visits reduces malaria-associated adverse outcomes including low birthweight (LBW). However, routine diagnostics miss low-density infections and IPTp-SP coverage remains suboptimal in many endemic settings. We conducted a prospective cohort study (2023-2024) at a medical health centre in Koubri, Burkina Faso. Women were enrolled at their first ANC visit, where *Plasmodium* infection was assessed by real-time polymerase chain reaction (PCR), microscopy, and rapid diagnostic test (RDT). IPTp-SP doses were given according to national guidelines. We used modified Poisson regression with robust variance to identify predictors of PCR-confirmed infection at first ANC visit and factors associated with LBW. Among 515 enrolled women, malaria prevalence at first ANC was 30.1% by PCR. In the subset of 183 women with all three tests available, prevalence was 23.5% by PCR versus 8.2% by microscopy and 8.7% by RDT, with approximately 2/3 of PCR-confirmed infections undetected by routine diagnostics. Primigravidae had twice the infection risk of multigravidae, and women living more than 5 km from the facility had higher risk. Each additional IPTp-SP dose was associated with an 11% reduction in PCR-confirmed infection prevalence during pregnancy. PCR-confirmed infection at first ANC was associated with a 52% higher risk of LBW. *Pfdhfr/Pfdhps* genotyping revealed a high prevalence of the quadruple mutant N51I+C59R+S108N_437G (66%), while the 540E and 581G mutations, associated with SP treatment failure, were each detected in only 3.4% of isolates, suggesting that SP resistance is not yet a primary driver of prophylaxis failure in this setting. In this Burkina Faso setting, routine IPTp-SP retained a measurable protective effect under routine conditions, but coverage gaps limited its population-level impact. The residual burden of malaria in pregnancy is driven more by implementation failures than by drug failure.

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IMPACT OF SATURATED CO-DEPLOYMENT OF IRS AND ITNS ON MALARIA RISK AND VECTOR ABUNDANCE IN HOLOENDEMIC NCHELENGE DISTRICT, ZAMBIA

Austin Earley[1], Westone Phanuel Hamwata[2], James Sichivula[2], Mike Chaponda[2], Mbanga Muleba[2], Mary Gebhardt[1], Anne Martin[1], Sophie Berube[1], Amy Wesolowski[1], Douglas E. Norris[1], Matthew Michael Ippolito[3], Jean-Bertin Bukasa Kabuya[2], William J. Moss[1]

[1]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [2]National Health Research & Training Institute, Ndola, Zambia, [3]Johns Hopkins University School of Medicine, Baltimore, MD, United States

Malaria transmission in Nchelenge District, located in northern Zambia on the border with the Democratic Republic of the Congo, has remained holoendemic despite having previously achieved near-universal ITN coverage and implementing targeted IRS campaigns. During 2012–2021, annual parasite prevalence by RDT averaged 25-50%, with peaks up to 75%. The reasons for persistently high transmission remain unclear, although it likely results from a combination of complex vector bionomics with *An. funestus* and *An. gambiae*, outdoor and early biting behaviors, housing quality, and population mobility. In 2023, public health partners conducted a co-deployment campaign of ITNs and IRS. To measure the impact of this campaign, the Southern Africa International Center of Excellence for Malaria Research established a longitudinal cohort in Nchelenge District, conducting monthly visits to 438 individuals in 65 households from October 2023 to September 2025. Sociodemographic characteristics, clinical history, ITN use, IRS status, and travel histories were collected. Participants were tested for malaria by RDT and microscopy. Entomological surveillance using CDC light traps was conducted at each visit to speciate and estimate mosquito abundance, and a subset of households was selected for human landing catches (HLC). Preliminary findings indicate that self-reported individual-level ITN use in the cohort increased from 42% pre-distribution to 91% on average over the course of the cohort. Household IRS coverage averaged 70% (54–82%). No significant reduction in parasite prevalence or *Anopheles* abundance was observed. Mean monthly parasite prevalence by RDT was 40% (26–56%) over the study period and was highest in children <15 years old (50%; 37–73%). HLC data revealed both indoor and outdoor biting, with peaks between 02:00–06:00 and 18:00–21:00, respectively. Despite saturated co-deployment of ITNs and IRS, stable transmission persists in this setting. Sustained and complementary interventions targeting outdoor and early biting vectors may help achieve measurable reductions in malaria transmission.

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COW-DERIVED ULTRALONG ANTIBODIES: A NEW FRONTIER IN MALARIA PREVENTION AND *PLASMODIUM* TRANSMISSION BLOCKING

Emily Bencosme-Cuevas[1], Tae Kwon Kim[2], Huldah Sang[3], Rakshith Kumar[3], Jayden McCall[3], Waithaka Mwangi[3], Joel Vega-Rodriguez[1]

[1]Molecular Parasitology and Entomology Unit, Laboratory of Malaria and Vector Research, NIAID, National Institutes of Health, Rockville, MD, United States, [2]HIV-Specific Immunity Section, Laboratory of Immunoregulation, NIAID, National Institutes of Health, Bethesda, MD, United States, [3]Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS, United States

Plasmodium falciparum, the most significant malaria parasite, is transmitted through the bite of infected *Anopheles* mosquitoes whose saliva facilitates hematophagy and parasite establishment via immune modulation. Monoclonal antibodies (mAbs) are being developed to block malaria transmission by targeting parasite and mosquito epitopes, but the limited and short-lived protection of current vaccines highlights the need for new interventions. Our team adopted an innovative approach that employs cow ultralong antibodies (CUAbs), which possess unusually long CDR H3 regions, enabling access to hidden or structurally complex epitopes

that may be inaccessible to traditional mAbs. To harness this structural advantage, cows were individually hyperimmunized with mosquito and parasite antigens: (1) anopheline salivary apyrase (apy), essential for *Plasmodium* mosquito infection and sporozoite transmission, and (2) *Plasmodium* CSP, a key mediator of hepatocyte invasion and primary vaccine target. Seroconversion was confirmed by ELISA, IgG were purified, and prophylactic & transmission-blocking activity was evaluated *in vitro* and *in vivo*. IgG against each antigen exhibited a reduction in different stages of the parasite: oocyst in mosquito midguts (apy-IgG), and sporozoites in liver cells (CSP-IgG). We next generated human-cow chimeric mAbs containing human constant region (backbone), while maintaining the cow antigen-binding regions. To achieve this, antigen-specific cow B cells were isolated by FACS, antibody genes were amplified by RT-PCR, cloned into expression vectors containing human constant regions, and subsequently expressed in human cell lines. These chimeric mAbs are under active evaluation for their prophylactic and transmission-blocking activity against the parasite and to define protective epitopes. This work introduces a scalable and versatile antibody platform that can complement existing malaria interventions and advance the development of effective, accessible prophylactic and transmission-blocking strategies.

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DURATION AND ASSOCIATION WITH PROTECTION OF NANP- AND C-TERMINUS-SPECIFIC ANTI-CIRCUMSPOROZOITE PROTEIN IGG RESPONSES FOLLOWING RTS,S/AS01$_E$ VACCINATION: AN ANCILLARY IMMUNOLOGICAL STUDY OF A PHASE 3 CLINICAL TRIAL

Maria Lara-Escandell[1], Lina Sánchez[1], Dídac Macià[1], Chenjerai Jairoce[2], Maxmillian Mpina[3], Selidji Todagbe Agnandji[4], David Dosoo[5], Ben Gyan[6], Marta Vidal[1], Alfons Jiménez[1], Robert Mitchell[1], Daniel Medina-Expósito[1], Laura Puyol[1], Rebeca Santano[1], Sheetij Dutta[7], Seth Owusu-Agyei[5], Kwaku Poku Asante[5], Halidou Tinto[8], Pedro Aide[2], Augusto Nhabomba[2], Benjamin Mordmüeller[9], Claudia Daubenberger[10], Ruth Aguilar[1], John Njenga Waitumbi[11], Gemma Moncunill[1], Carlota Dobano[12]

[1]Barcelona Institute for Global Health - ISGlobal, Barcelona, Spain, [2]Manhiça Health Research Center (CISM), Maputo, Mozambique, [3]Biomedical Research and Clinical Trials Department, United Republic of Tanzania, [4]Centre de Recherches Médicales de Lambaréné (CERMEL), Lambarene, Gabon, [5]Kintampo Health Research Centre, Research & Development Division, Ghana Health Service, Kintampo, Ghana, [6]Noguchi Memorial Institute for Medical Research, Accra, Ghana, [7]WRAIR, Silver Spring, MD, United States, [8]Unité de Recherche Clinique de Nanoro, Institut de Recherche en Sciences de la Santé, Nanoro, Burkina Faso, [9]Department of Medical Microbiology, Radboud University Medical Center, Nijmegen, Netherlands, [10]Swiss Tropical and Public Health Institute, Allschwil, Switzerland, [11]USAMRU-K, Kisumu, Kenya, [12]Barcelona Institute for Global Health (ISGlobal), Barcelona, Spain

The RTS,S/AS01$_E$ malaria vaccine provides partial protection against *Plasmodium falciparum*, largely mediated by antibodies to the circumsporozite protein (CSP). Correlates of protection remain incompletely defined and have focused mainly on peak IgG responses to the immunodominant NANP-repeats, with less known about antibody durability or responses to the C-terminal of CSP. In this observational study, we evaluated IgG responses to NANP-repeat, C-terminal and full-length CSP constructs at five timepoints before and after primary and booster vaccination, in 1,292 children aged 5-17 months from six African countries enrolled in the RTS,S/AS01$_E$ phase 3 trial (NCT00866619, 2009-2011). Antibody kinetics were analyzed with linear regressions, and associations with clinical malaria risk over one year with Cox models. IgG levels declined more slowly for C-terminal than for NANP-repeat over 17-months after primary vaccination (geometric mean fold-change [GM FC]: 0.099 [95%CI 0.089-0.11] vs 0.055 [95%CI 0.048-0.062]; p<0.0001). The booster significantly enhanced C-terminal IgG, exceeding post-primary peak levels (GM FC 1.98 [95%CI 1.77-2.21]), whereas NANP-repeat responses were restored but remained below post-primary levels (GM FC 0.57 [95%CI 0.51-0.63]). Higher CSP IgG levels correlated with reduced malaria risk, particularly during maintenance phases, with stronger associations for C-terminal, which remained independently protective after adjustment for NANP-repeat antibodies (HR=0.60, 95%CI 0.43-0.83, p=0.002). After boosting, maintenance but not peak IgG levels correlated with reduced malaria risk. IgG to CSP C-terminal are more durable than those to the NANP-repeats. Antibody maintenance emerges as a stronger predictor of RTS,S/AS01$_E$ vaccine efficacy than peak responses.

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RTS,S MALARIA VACCINE IMMUNE RESPONSES ARE ADVERSELY IMPACTED BY *PLASMODIUM FALCIPARUM* GENETIC DIVERSITY.

Alessia Hysa[1], Liriye Kurtovic[1], D. Herbert Opi[1], Myo Naung[1], Alyssa Barry[2], Alassane Dicko[3], Jean-Bosco Ouedrago[4], Daniel Chandramohan[5], Brian Greenwood[6], James Beeson[1]

[1]Burnet Institute, Melbourne, Australia, [2]Deakin University, Geelong, Australia, [3]RTS,S SMC clinical trial NCT04319380 team, Bamako, Mali, [4]RTS,S SMC clinical trial NCT04319380 team, Bobo-Dioulasso, Burkina Faso, [5]RTS,S SMC clinical trial NCT04319380 team, London, United Kingdom, [6]RTS,S SMC clinical trial NCT04319380 team, Hertfordshire, United Kingdom

The RTS,S malaria vaccine provides modest and short-lived efficacy against malaria in children (~55% over 12-months). RTS,S targets the *Plasmodium falciparum* circumsporozoite protein (CSP) of one parasite strain (3D7) which represents less than 10% of the circulating African parasite population. In malaria endemic regions, parasite isolates present a range of CSP sequences on their surface that vary to the 3D7 strain by few amino acid changes and there is no dominant CSP sequence. Vaccine-induced immune responses to variant non-3D7 CSP sequences remain severely understudied, but CSP diversity may result in lower vaccine efficacy and parasite immune escape. We compared RTS,S-induced antibody binding (IgG) and Fc-mediated functional responses (C1q complement fixation and FcγRIII binding) between the vaccine CSP 3D7 strain and four variant non-3D7 CSP strains prevalent in naturally occurring African parasite isolates. The non-3D7 CSP sequences varied to the vaccine 3D7 CSP sequence by 4-9 amino acid differences. We measured responses in a large RTS,S phase 3 clinical trial in young children 5-17 months old (n=1900) and found reduced IgG binding to non-3D7 strains compared to 3D7. Interestingly, this reduction was more pronounced when we evaluated Fc-mediated functional activities, suggesting polymorphisms impact antibody function more so than IgG antibody recognition. Furthermore, RTS,S-induced antibody functional activity was lowest to the most diverse CSP sequences that varied to the vaccine 3D7 sequence by 8 and 9 amino acid changes. Our findings suggest that naturally occurring CSP diversity adversely impacts vaccine-induced immune responses and this may explain why RTS,S provides modest protection in endemic regions.

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THE MONOCLONAL ANTIBODY L9LS IS SAFE AND EFFICACIOUS AGAINST MALARIA IN INFANTS BUT INTERFERES WITH THE R21/MATRIX-M MALARIA VACCINE

Bickey H. Chang[1], Kassoum Kayentao[2], Aissata Ongoiba[2], Anne C. Preston[1], Sara A. Healy[1], Zonghui Hu[3], Jeff Skinner[1], Safiatou N. Doumbo[2], Jing Wang[4], Hamidou Cisse[1], Didier Doumtabe[2], Abdrahamane Traore[2], Hamadi Traore[2], Adama Djiguiba[2], Shanping Li[1], Mary E. Peterson[1], Sandeep Narpala[5], Bob C. Lin[5], Leonid Serebryannyy[5], Siriman Traore[2], Mamadou Keita[2], Amatigue Zeguime[2], Adama Ouattara[2], M'Bouye Doucoure[2], Amagana Dolo[2], Boubacar Traore[2], Robert A. Seder[5], Peter D. Crompton[1]

[1]Malaria Infection Biology and Immunity Section, Laboratory of Immunogenetics, Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States, [2]Malaria Research and Training Center, Mali International Center of Excellence in Research, University of Sciences, Techniques, and Technologies of Bamako, Bamako, Mali, [3]Biostatistics Research Branch, Division of Clinical Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States, [4]Clinical Monitoring Research Program Directorate, Frederick National

Laboratory for Cancer Research, Frederick, MD, United States, [5]Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States

Infants are not eligible for the WHO-recommended malaria vaccines RTS,S/AS01 and R21/Matrix-M until 5 months of age, and vaccine-mediated protection is not fully established until 3 months later, leaving young infants vulnerable to malaria. L9LS is a long-acting monoclonal antibody (mAb) targeting a conserved epitope of the *Plasmodium falciparum* circumsporozite protein (PfCSP) that has shown efficacy against malaria in older children in Phase 2 trials in Mali and Kenya. Although L9LS primarily targets an epitope on PfCSP not contained in the RTS,S or R21 vaccines, it binds R21 weakly in vitro, raising the possibility that it could interfere with the immunogenicity of the R21 vaccine. We conducted a phase 1b trial in Mali to assess the safety and efficacy of intramuscular administration of L9LS in healthy infants 1 to 12 months of age over a 6-month malaria season, followed by an assessment of the impact of L9LS on the immunogenicity of subsequent R21 vaccination. One hundred eighty infants were randomized 1:1 to receive 150 mg of L9LS or placebo before the malaria season, followed by the 3-dose R21 primary vaccine series 5 months later. We found that L9LS was safe, well tolerated, and exhibited expected pharmacokinetics in serum. The protective efficacy of L9LS as compared with placebo was 78% (95% confidence interval 36-93%) against *P. falciparum* infection during the 6-month malaria season. We observed a decrease in R21 immunogenicity as measured by total NANP-specific IgG titers in infants who received L9LS compared to those who received placebo. Our study demonstrates that L9LS is safe and efficacious in infants; however, L9LS-mediated interference with R21 may limit its use to populations who have already received the R21 vaccine or who are not eligible for R21 vaccination (children older than 3 years of age and adults). The ongoing development of anti-malaria mAbs that target epitopes not included in the R21 and RTS,S vaccines offers a promising alternative for protecting young infants from malaria.

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A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED PHASE 1 TRIAL TO EVALUATE THE SAFETY, TOLERABILITY, IMMUNOGENICITY, AND EFFICACY OF SANARIA PFSPZ-LARC2 VACCINE BY CONTROLLED HUMAN MALARIA INFECTION IN MALARIA-NAÏVE HEALTHY ADULTS

Sean C. Murphy[1], Lauren Jatt[2], James Kublin[3], Kirsten Hauge[1], Jessica Heimonen[1], Tara M. Babu[1], Kaniz Khan[4], William Sears[5], Kaylyn Brock[6], Jordan Lundeen[6], Ranjodh Gill[7], James Campbell[8], B. Kim Lee Sim[9], Thomas Laurence Richie[9], Stephen L. Hoffman[10], R. Scott McClelland[1]

[1]Univ of Washington, Seattle, WA, United States, [2]Seattle, WA, United States, [3]Fred Hutch, Seattle, WA, United States, [4]National Institutes of Health, Rockville, MD, United States, [5]Silver Spring, MD, United States, [6]The Emmes Corporation, Frederick, MD, United States, [7]Rockville, MD, United States, [8]University of Maryland, Baltimore, MD, United States, [9]Sanaria Inc., Rockville, MD, United States, [10]Sanaria Inc, Rockville, MD, United States

A highly effective malaria vaccine is needed to protect children and other at-risk populations including travelers and the military and to accelerate global malaria elimination efforts. Radiation-attenuated, aseptic, purified, cryopreserved *Plasmodium falciparum* (Pf) sporozoites (PfSPZ) administered by direct venous inoculation (DVI) have protected human volunteers against controlled human malaria infection (CHMI) but greater efficacy and durability are desired. Whereas irradiation introduces random mutations, gene deletion can be used to create genetically attenuated parasites (GAP) that are molecularly defined, are cloned to assure uniformity, and do not cause blood-stage infection. To harness the improved immunogenicity induced by mature liver stages while minimizing the risk of breakthrough blood-stage infection, late-arresting, replication competent (LARC) GAPs were developed. PfSPZ-LARC2 Vaccine is a LARC GAP genetically modified by markerless deletion of *Mei2* and *LINUP* genes that is well supported by pre-clinical studies. Starting with a sentinel cohort (3 vaccinated, 1 saline placebo) and proceeding one month later to enrolment of a larger cohort (12 vaccinated, 6 saline placebo), 22 participants underwent DVI with either $2x10^5$ PfSPZ-LARC2 Vaccine or placebo three times at 28-day intervals followed by CHMI by DVI of heterologous PfSPZ of PfSPZ-Challenge (7G8) 12 weeks (main cohort) or 16 weeks (sentinel cohort) after the last immunizing dose. Thick blood smears (TBS) and Pf 18S rRNA reverse transcription PCR (RT-PCR) were performed frequently throughout post-vaccination and CHMI visits to monitor infection status. Blood was collected to assess innate, humoral, and cellular immune responses. Unblinded vaccine efficacy against heterologous CHMI and blinded safety data will be presented.

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EFFECTIVENESS AND SAFETY OF THE RTS,S/AS01E MALARIA VACCINE ONE YEAR AFTER THE FULL-SCHEDULE COMPLETION IN REAL-LIFE SETTINGS IN THREE SUB-SAHARAN AFRICAN COUNTRIES: FINAL ANALYSIS

Bernhards Ogutu Ragama[1], and the RTS,S Epidemiology EPI-MAL-003 study group[2]

[1]Kenya Medical Research Institute, Centre for Clinical Research, Kisumu, Kenya, [2]Kenya

As part of the WHO-coordinated Malaria Vaccine Implementation Programme, RTS,S/AS01$_E$ was introduced into national immunization programs in selected areas in Ghana, Kenya and Malawi. EPI-MAL-003 (NCT03855995) is a prospective surveillance study conducted between 2019 and 2025, to assess safety, effectiveness and impact of RTS,S/AS01$_E$ in children under 5 years of age. Children were followed up through 10 home visits and continuous monitoring of outpatient visits and hospitalizations in exposed (where the vaccine had been introduced) and unexposed clusters; data were collected via active surveillance. This analysis included 44,912 children (exposed clusters: 22,448; unexposed clusters: 22,464). In the exposed clusters, the fourth RTS,S/AS01$_E$ dose coverage was 64%. The total effect of the RTS,S/AS01$_E$ vaccination from dose 3 up to 1 year post-dose 4 showed reductions of 14% in any malaria (incidence rate ratio [IRR]: 0.86, 95% confidence interval [CI]: 0.84-0.88, p<0.001), 27% in severe malaria cases (IRR: 0.73, 95% CI: 0.58-0.92, p=0.009), 22% in malaria-related hospitalizations (IRR: 0.78, 95% CI: 0.72-0.86, p<0.001), and 23% in anemia among hospitalized children (IRR: 0.77, 95% CI: 0.71-0.84, p<0.001). The reductions of 9% in all-cause mortality (IRR: 0.91, 95% CI: 0.58-1.43, p=0.687) and 6% in malaria-related mortality (IRR: 0.94, 95% CI: 0.44-2.03, p=0.876) were not statistically significant. Up to one year after the full vaccination schedule, the risk was similar between vaccinated and unvaccinated children for etiology confirmed meningitis (IRR: 1.06, 95% CI: 0.07-16.98, p=0.966) and cerebral malaria (IRR: 0.76, 95% CI: 0.24-2.39, p=0.637). From dose 3 up to 1 year post-dose 4 in real-life settings, RTS,S/AS01$_E$ significantly reduced the incidence of malaria, severe malaria, malaria-related hospitalizations and anemia. Overall, the benefit-risk profile of RTS,S/AS01$_E$ remained favorable. These findings support continued use of RTS,S/AS01$_E$ for malaria prevention and control in children under 5 years of age in endemic settings.

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CASE-CONTROL STUDIES OF THE EFFECTIVENESS OF THE RTS,S/AS01 MALARIA VACCINE AGAINST SEVERE MALARIA

Thomas Gyan[1], Ari Fogelson[2], Samuel Owuor Akech[3], Don Pascal Mathanga[4], Christopher Chikhosi Stanley[4], Titus Kimutai Kwambai[5], Elisha Adekunle Adeniji[1], Cynthia Khazenzi[6], Harrison Msuku[7], Paul Snell[2], Augustine Sarfo[1], Stephaney Gyaase[1], Aubrey Kamanga[8], Irene Nailain Nkumama[9], Simon Kariuki[10], Samuel Ekow Bernard Harrison[1], Monica Musa[6], Gordon Orwa[11], Tisungane Mvalo[12], Yvette Collymore[13], Mary J. Hamel[14], Rafiq Nii Attoh Okine[14], Paul John Milligan[2], Kwaku Poku Asante[1]

[1]Kintampo Health Research Centre, Kintampo, Ghana, [2]London School of Hygiene&Tropical Medicine, London, United Kingdom, [3]US CDC Kenya, Nairobi, Kenya, [4]Kamuzu University of Health Sciences, Blantyre, Malawi,

[5]American Centers for Disease Control and Prevention-Kenya, Kisumu, Kenya, [6]KEMRI-Wellcome Trust Research Programme, Nairobi, Kenya, [7]Malaria Alert Center, Blantyre, Malawi, [8]Malaria Alert Centre, Kamuzu University of Health Sciences, Blantyre, Malawi, [9]European Vaccine Initiative (EVI), Heidelberg, Germany, [10]Kenya Medical Research Institute, Kisumu, Kenya, [11]Kenya Medical Research Institute, Centre for Global Health Research, Kisumu, Kenya, Kisumu, Kenya, [12]UNC, Lilongwe, Malawi, [13]PATH Malaria Vaccine Initiative, Seattle, WA, United States, [14]World Health Organization, Geneva, Switzerland

Case-control studies were undertaken during and shortly after the Malaria Vaccine Pilot Evaluation, to determine the incremental benefit of 4 doses of RTS,S vaccine compared to 3 doses when implemented through routine child immunization programmes in Ghana, Kenya and Malawi, to assess evidence for any rebound effect in children who received 3 doses and not the fourth dose, and to estimate associations with safety signals that had been observed in the phase3 trial. Four neighbourhood controls born within 1 month of the case were recruited for each case. Caregivers were visited at home to collect information on vaccination history and potential confounders. Dates of vaccination with RTS,S and other vaccines were copied from the child's health booklet or vaccination record, or from clinic registers. Records were photographed for verification later. The index date for vaccination status in the controls was the date of the case event (for safety signals), or 14 days before the event (for estimating effectiveness). Conditional logistic regression was used to estimate odds ratios adjusted for covariates. A total of 1546 cases of severe malaria were recruited, with 6151 controls. The average effectiveness of 3 doses of RTS,S, during the period until the 4th dose was due, was 56% (95%CI 40%,67%), an interval of up to 15 months. Among children who did not go on to receive the 4th dose, the average effectiveness of 3 doses from the age when the 4th dose was due was 35% (95%CI 15%,50%). Among children who received the 4th dose, the average effectiveness of 4 doses against severe malaria was 54% (95%CI 39%, 66%). The incremental effectiveness of dose 4, compared to 3 doses, was 30% (95%CI 9.3%,45%). The protection associated with 3 doses of RTS,S persisted beyond the age when children were due to receive dose 4. There was no evidence of a rebound effect. The 4th dose provided substantial additional protection lasting at least 18 months. RTS,S was as effective against cerebral malaria as for other forms of severe malaria. We found no evidence that RTS,S was associated with an excess risk of meningitis, and no evidence that the gender modified the effect of RTS,S on mortality.

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DIRECT SKIN FEEDING ENDPOINTS FOR MALARIA TRANSMISSION BLOCKING VACCINE TRIALS

Gail Potter[1], Dean Follmann[1], Patrick Emmet Duffy[2]

[1]National Institutes of Health, Rockville, MD, United States, [2]National Institutes of Health, NIAID/Lab of Malaria Immunology & Vaccinology, Bethesda, MD, United States

Malaria causes tremendous morbidity and mortality, with 282 million cases and over 600,000 deaths worldwide in 2024. Transmission blocking vaccines (TBV) aim to interrupt transmission of malaria parasites from humans to mosquitoes, thus reducing the reservoir that can infect humans in the community. Cluster-randomized trials are needed to estimate TBV clinical benefits, but these are often infeasible for early phase trials. Instead, feeding assays are used to measure individual human-to-mosquito parasite transmission. In the Direct Skin Feeding (DSF) assay, multiple mosquitoes feed directly on the skin of humans. Mosquitoes surviving a week are dissected, and their midguts are examined for malaria oocysts. The DSF recapitulates natural human transmission of locally circulating malaria parasites to locally relevant mosquito species as well as the vaccine mechanisms that kill parasites in the mosquito midgut. Trial participants undergo serial DSF testing during the transmission season, providing longitudinal data. We compare three DSF efficacy endpoints: "VE mosquito" = the vaccine-induced reduction in proportion of infected mosquitoes (across all DSFs); "VE DSF" = the reduction in proportion of DSFs with transmission to at least one mosquito; and "VE person" = the reduction in proportion of participants transmitting at least once. We demonstrate that VE mosquito best predicts community-wide effectiveness in reducing clinical malaria by introducing the concept of "VE visitor", the reduction in probability that a village visitor becomes ill with malaria if the village is TBV-vaccinated vs. unvaccinated. We show that VE mosquito estimates VE visitor, while VE person and VE DSF underestimate it. We present results from two clinical trials validating these relationships. With a simulation model calibrated to the trial data, we show that VE mosquito is the most powerful, requiring half the sample size of VE person in many scenarios. While added power is a bonus, the reason that VE mosquito is the best early phase surrogate is that it can best differentiate which early phase vaccine is most likely to reduce clinical malaria in the community.

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MALARIA TRENDS IN CHILDREN UNDER FIVE FOLLOWING RTS,S/AS01 INTRODUCTION IN HIGH-TRANSMISSION DISTRICTS OF BURKINA FASO

Yacouba Belem[1], Cheick Compaore[2], Franck Palamanga Obulbiga[3], René Didace Bakouan[4], Zongo Youssouf[1], Soumeya A.S. Ouangraoua[5], Amos Asiedu[6], Gladys Tetteh[7], Sidibe Anissa[8], Christelle Neya[9]

[1]Jhpiego, Ouagadougou, Burkina Faso, [2]Malaria Consortium, Ouagadougou, United States, [3]Jhpiego Corporation Burkina Faso, [4]bakouan, Seattle, WA, United States, [5]Centre Muraz Unit of Nutrition Evnironment & Prone Epidemic Diseases, Bobo-Dioulasso, Burkina Faso, [6]Accra, Ghana, AA, Ghana, [7]Jhpiego, Washington, DC, United States, [8]Jhpiego, Baltimore, MD, United States, [9]EPI, Ouagadougou, Burkina Faso

Since the WHO recommended malaria vaccines in 2021, 25 African countries have introduced RTS,S/AS01 or R21/Matrix-M with Gavi support. As countries simultaneously scale up seasonal malaria chemoprevention (SMC), insecticide-treated nets (ITNs), and case management, understanding the incremental impact of vaccination is essential for guiding resource allocation. Burkina Faso introduced RTS,S/AS01 on 5 February 2024 across 27 high-transmission districts, targeting ~250,000 children aged 5-23 months. By December 2025, dose 1-4 coverage reached 77%, 72%, 67%, and 52%. Using DHIS2 data, we compared pre-introduction (February 2022-January 2024) and post-introduction (February 2024-January 2026) confirmed cases, hospitalizations, and deaths among children under five. Vaccinating districts were stratified by dose-4 coverage: high (≥68%, n=6) and low (<68%, n=21). Trends were also examined across 43 non-vaccinating districts. High-coverage districts showed reductions of 46% in cases, 26.7% in hospitalizations, and 54.2% in deaths; low-coverage districts also showed reductions of 36.5%, 23.2%, and 22.7%. Non-vaccinating districts also experienced 30%, 16.7%, and 40.3% reductions, suggesting broader control intensification - SMC expansion, ITN campaign, destruction of mosquito breeding sites during the SMC, strengthening community-based malaria care, drove much of the decline. The greater death reduction in high-coverage vaccine districts (54.2% vs. 22.7% in low-coverage and 40,3% in non-vaccinating districts) may indicate a vaccine effect on severe disease progression. Limitations include inability to verify equivalent intervention coverage across groups, and that non-vaccinating districts were indeed lower-transmission areas. These findings illustrate that ecological analyses of routine data face too many confounding factors to isolate vaccine-specific impact. Prospective cohort studies comparing vaccinated and unvaccinated children within the same settings are needed to quantify vaccination's independent contribution, evidence essential as endemic countries prioritize limited resources.

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IMPACT OF VIRUS GENETIC DIVERSITY AND EVOLUTION ON INHIBITION OF DENGUE VIRUS BY *WOLBACHIA* IN *AEDES AEGYPTI*

Julian W. Bakker[1], Nicole M. Feriancek[1], Afeez Sodeinde[1], Angkana T. Huang[2], Chantal B.F. Vogels[1]

[1]Yale University, School of Public Health, New Haven, CT, United States, [2]NUS Saw Swee Hock School of Public Health, Singapore, Singapore

The mosquito-borne dengue virus is expanding worldwide, posing a growing threat to global public health. A promising control strategy is the release of *Aedes aegypti* mosquitoes transinfected with *Wolbachia*, an endosymbiotic bacterium that suppresses dengue virus transmission. This biological intervention has already been implemented in multiple countries and has demonstrated substantial impact. However, *Wolbachia*-mediated inhibition may be incomplete, and consistent reductions in dengue transmission are not always achieved. Furthermore, dengue viruses are genetically diverse, rapidly replicating RNA virus, raising concerns that it may adapt and possibly escape from *Wolbachia*-based control. An important outstanding question is whether dengue virus could evolve to overcome the inhibitory effects imposed by *Wolbachia* in mosquitoes. Here, we present the most comprehensive study to date to address whether dengue virus genetic diversity allows for escape from *Wolbachia*-mediated inhibition. We found that the *Wolbachia w*AlbB and *w*MelM strains, transinfected into *Ae. aegypti*, vary in their ability to inhibit 60 genetically diverse dengue virus isolates spanning all four serotypes, with more DENV-3 strains that were weakly inhibited. From this dataset, we identified candidate mutations that may be associated with possible *Wolbachia* escape. In parallel, to expand our understanding of the virus genetic determinants underlying potential escape, we applied a deep mutational scanning approach. With this method, we generated viral libraries containing nearly all possible amino acid substitutions in the envelope protein across all four dengue serotypes. We then engineered selected mutations identified through both approaches into dengue virus clones representing each serotype and evaluated their functional effects using high-throughput phenotypic assays. Our findings suggest that the genetic diversity of dengue virus may influence the long-term success and durability of these interventions, an important consideration with the continued global scaling of *Wolbachia*-based interventions.

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EVOLUTION OF THE WEST NILE VIRUS ENVELOPE PROTEIN ACROSS ONE HUNDRED YEARS

Jack Dorman, Patrick Dolan

NIH, Bethesda, MD, United States

The structural proteins of flaviviruses are subjected to complex selective pressures given they balance fitness in vertebrate and arthropod hosts. Of these pathogens, West Nile virus (WNV) is the most common in the United States. Recent results have demonstrated adaptation to different hosts as the virus has spread globally, but these findings only extend to very recent evolutionary history since the advent of public health surveillance. To better understand how adaptation has occurred over longer timescales, we applied ancestral sequence reconstruction to a key WNV protein, the Envelope (E) protein extending across the recent century of evolution. We produced all 184 intermediate sequences reaching from a common ancestor to multiple contemporary strains. Performing a competition assay on mosquito, and bird cells as well as human neurons, we demonstrated that as these ancestral viruses accumulated historical mutations, the lineage that went on to cause a large outbreak in the Americas increased fitness in bird and mosquito cells relative to Afro-Eurasian isolates. Further, ancestral fitness is preserved across most derived genotypes with the exception of select terminal isolates that often have decreased fitness on bird or mosquito cells. However, most isolates have increased their human neurotropism relative to the ancestral E. Our results have systematically demonstrated the dynamic nature of host preference over recent evolutionary history. Such insights will improve our understanding of how novel genotypes have previously emerged and how future genotypes may arise.

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DIVERGENT EVOLUTIONARY PATTERNS OF CRIMEAN CONGO HEMORRHAGIC FEVER VIRUS EXIST IN EAST AFRICA

Maria Gorreti Onyango[1], Kwasi Baako[1], Victor Okoth Anyango[2], Winnifred Aool[1], Lucia Kiio[2], Martin Lukindu[3], Teddy Nakayaki[4], Julius Julian Lutwama[4], Jonathan Kayondo[4], Joel Lutomiah[5], James Kamami Mutisya[6], Corey Brelsfoard[1]

[1]Texas Tech University, Lubbock, TX, United States, [2]Lubbock, TX, United States, [3]Uganda Virus Research Institute, Entebbe, Kenya, [4]Uganda Virus Research Institute, Entebbe, Uganda, [5]Kenya Medical Research Institute (KEMRI), Nairobi, Kenya, [6]Kenya Medical Research Institute (KEMRI), NAIROBI, Kenya

Crimean-Congo hemorrhagic fever virus (CCHFV) is a widespread tick-borne virus with high mortality in humans. In East Africa, a significant epidemiological disparity exists between two countries that share an extensive border: Uganda reports recurrent outbreaks, whereas Kenya has never reported a confirmed outbreak despite high serologic evidence of exposure. This paradox remains unresolved largely due to the lack of comprehensive CCHFV genomic data from Kenya. This study addresses this critical gap by generating and analyzing one of the first complete CCHFV genomes from Kenya and comparing it with regional and global strains.To assess evolutionary pressures, we analyzed phylogenetic relationships, GP38 mutations and codon usage metrics of CCHFV isolates obtained from tick pools collected from the Ugandan cattle corridor and Northern Kenyan pastoralist region.Tick communities varied by region: Kenyan sites were dominated by *Hyalomma* ticks with low diversity, while Ugandan sites showed more diverse, *Rhipicephalus*- assemblages. Phylogenetically, the Kenyan isolate represents a stable, basal lineage linked to historical southern African strains. Conversely, Ugandan isolates belonged to a diverse, outbreak-associated lineage connected to Central/West African and Asian strains. Structural mapping identified significant conservation in Kenyan strain GP38 protein. Ugandan mutations primarily clustered in the GP38 N-terminal three-helix bundle and β-sandwich loops. Codon usage analysis revealed low bias, supporting a generalist evolutionary strategy driven by mutational pressure.We propose that the localized diversification of the GP38 N-terminal three-helix bundle and β-sandwich loops in Ugandan isolates function as an immunological decoy ensuring the protein's successful secretion, resulting in vascular leakage and severe clinical manifestations. Conversely, the structural conservation of the ancestral Kenyan lineage may enhance its immunogenicity, leading to subclinical infections that contribute to population immunity against more virulent CCHFV strains.

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THE ECOLOGICAL AND MOLECULAR DRIVERS OF REASSORTMENT IN ORTHOHANTAVIRUSES

Ricardo Rivero[1], David Simons[2], Lambodhar Damodaran[3], Daniel Becker[4], Dan L. Warren[5], Nicola F. Müller[6], David Rasmussen[7], Stephanie Seifert[1]

[1]Washington State University, Pullman, WA, United States, [2]The Royal Veterinary College, Stockholm, AB, Sweden, [3]Durham, NC, United States, [4]Norman, OK, United States, [5]Charles Sturt University, Wagga Wagga, Australia, [6]University of California San Francisto, San Francisto, CA, United States, [7]North Carolina State University, Raleigh, NC, United States

The evolutionary history of hantaviruses is shaped by host switching and segment reassortment, rather than strict codivergence with reservoir hosts. However, the ecological settings and genomic constraints that enable reassortment remain poorly understood. We compiled a hantavirus dataset with 15,362 sequences representing 66 taxa sampled across 236 host species. We linked these data to ArHa, a reservoir and prevalence resource with 917 studies and over 500,000 observations. We integrated

phylogenomic analyses with host traits, predicted host-range overlap, and segment compatibility metrics. After filtering, we identified 638 complete S/M/L genomic triplets across seven focal viral species. Reassortment events were identified using Espalier. We then applied machine learning to identify the ecological and molecular features that distinguished reassortant from non-reassortant genomes. Reassortment was not associated with any single ecological or genomic variable alone. Instead, support was strongest where ecological opportunity for co-circulation coincided with molecular compatibility among exchanged segments. Ecological opportunity was captured by host-range overlap estimated from species distribution models and ArHa localities, together with host phylogenetic breadth and life-history traits linked to movement and transmission, including dispersal, body size, among others. Molecular compatibility was reflected by tree discordance, non-random linkage among genomic sites, and 5' untranslated region structural features, consistent with constraints on whether reassorted segment combinations remain competent. Although the relative contribution of these factors varied across viral lineages, the pattern supported a model in which reassortment is most likely when host contact, co-infection opportunity, and segment compatibility align. These results advance understanding of hantavirus reassortment toward a mechanistic framework linking reservoir ecology, geographic overlap, and segment-level compatibility, with implications for surveillance and risk prioritization of zoonotic lineages.

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MOLECULAR CHARACTERIZATION OF MEASLES VIRUS IN THE DEMOCRATIC REPUBLIC OF CONGO

Francisca Muyembe-Mawete[1], Junior Bulabula-Penge[2], Catherine Pratt[3], François Edidi-Atani[4], Eddy Kinganda-Lusamaki[5], Patrick Mukadi-Kakoni[6], Fabrice Mambu-Mbika[7], Prince Akil-Bandali[5], Princesse Paku-Tshambu[5], Chloé Musuamba-Kayembe[5], Placide Kingebeni Mbala[6], Steve Ahuka-Mundeke[6], Jean-Jacques Muyembe-Tamfum[6], Kimihito Ito[1]

[1]Division of Bioinformatics, Graduate School of Infectious Diseases, Hokkaido University, Sapporo, Japan, [2]Ecoepidemiology and Epidemiological Informatics, Institute of Tropical Medicine,Nagasaki University, Nagasaki, Japan, [3]Biosurv International, Salisbury, United Kingdom, [4]Division of Global Epidemiology, International Institute for Zoonosis Control, Hokkaido University, Sapporo, Japan, [5]Institut National de Recherche Biomédicale, Kinshasa, Democratic Republic of the Congo, [6]Institut National de Recherche Biomédicale, Kinshasa University, Kinshasa, Democratic Republic of the Congo, [7]Graduate School of Biomedical Science, Institute of Tropical Medicine, Nagasaki University, Nagasaki, Japan

Measles remains a significant cause of mortality in Africa, with case fatality rates markedly higher than in industrialized countries. To support the World Health Organization (WHO) elimination strategies, this study updates the molecular characterization of the measles virus in the Democratic Republic of the Congo (DRC) using samples collected during national surveillance. Ethical approval for the genomic analysis of these samples was granted by the School of Public Health of Kinshasa (ESP/CE/56/2026). Between January 2022 and March 2023, 186 throat swabs and sociodemographic data were collected across 23 health zones of 13 provinces. Viral RNA was extracted, and the N gene was quantified via RT-qPCR. Of 186 samples, 131 (70.4%) were positive for measles virus. Positivity was significantly higher in children under 5 (p=0.008) and unvaccinated patients (p=0.0083). Samples with a Ct < 30 were sequenced using an Oxford Nanopore platform. Genomic sequencing yielded 61 high-quality C-terminal N gene sequences, all of which were identified as genotype B3. Kinshasa province provided the most sequences (n=17), with a nucleotide diversity of 0.0021. A positive correlation was observed between viral genetic distance and geographic distance between provincial capitals ($p<10^{-15}$), indicating that divergence increases with geographic distance. The outbreak was driven exclusively by the B3 genotype. These results suggest localized transmission networks, where geographically distant provinces exhibit greater viral nucleotide differences.

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WEST NILE VIRUS INFECTIONS ARE INITIATED BY COLLECTIVE INFECTIOUS UNITS

Marina Fujii, Emily Anne Fitzmeyer, Emily Gallichotte, Kate X. Kimball, Brian Geiss, Gregory D. Ebel

Colorado State University, Fort Collins, CO, United States

A virus infectious unit is widely considered to be a single virus particle that enters a cell to initiate the infectious cycle. However, a growing body of research suggests that most viral infections are initiated by multiple viral genomes that infect a given cell as a group. The presence of multiple virus genomes in the same cell has been termed 'polyinfection.' Polyinfection has important evolutionary implications, as the genetic composition of the infectious unit represents the material transmitted between hosts and therefore defines the effective transmission bottleneck, a key determinant of viral genetic diversity and evolution. To determine the extent of polyinfection in West Nile virus (WNV) infections, we assessed the genomic content of various WNV infectious units, including plaque-forming units, tissue culture infectious units, and single-cell infectious units, using a molecularly barcoded WNV. Our results reveal that polyinfection occurs across all types of infectious units, but to different extents: most plaque-forming units contain multiple viral genomes, whereas polyinfection is less frequent in tissue culture infectious units or single-cell infectious units. Additionally, we found that sonicating the virus solution prior to infection reduced the number of viral genomes per plaque-forming unit, indicating that multiple genomes are packaged within collective infectious units. To understand interactions between coinfecting viral genomes, we are currently examining complementary interactions using molecularly marked clones with engineered attenuating mutations.

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PHYLOGENETIC ANALYSIS OF CHIKUNGUNYA VIRUS ASSOCIATED WITH THE 2024-2025 OUTBREAK OF UNDIFFERENTIATED ACUTE FEBRILE ILLNESS IN KENYA'S COASTAL REGION

Beth Kinya Mutai[1], Gathii Kimita[2], Josphat Nyandieka Nyataya[1], Allan Poghisyo Lemtudo[3], Abdi Roba[4], John Njenga Waitumbi[5]

[1]KEMRI / WRAIR - Africa, Kisumu, Kenya, [2]KEMRI-WRAIR - Africa, Kisumu, Kenya, [3]KEMRI - WRAIR - Africa, kisumu, Kenya, [4]Ministry of Health Division of National Public Health Laboratories, Nairobi, Kenya, [5]KEMRI / WRAIR -Africa, Kisumu, Kenya

Introduction: Chikungunya virus (CHIKV) causes outbreaks of febrile illness, accompanied by severe joint pains. This study describes the phylodynamics of a CHIKV outbreak that was first reported northeastern Kenya in December 2024 and subsequently spread to coastal towns. Methodology: In December 2024, cases of febrile illness with headache and arthralgia were reported at Hagadera Refugee Camp (HRC) in Garissa County. From January to December 2025, similar cases appeared at Kenya's coastal towns of Mombasa and Lamu. Plasma samples (n = 34 from HRC outbreak) and 1383 from febrile patients at sentinel health facilities in Mombasa and Lamu were screened for CHIKV by qPCR. Positive samples from HRC were sequenced on the PromethION (Oxford Nanopore Technologies, UK). The coastal samples were enriched for virus using VirCapSeq-VERT probes and sequenced on Illumina NextSeq 2000. Spatiotemporal phylogenetic analyses was conducted using Nextstrain. Results: Of the 34 HRC samples and 1383 from the coastal region, 13 (38.2%) and 478 (34.6%) tested positive for CHIKV. 144 near-complete CHIKV genomes were recovered and all classified within the East/Central/South African lineage and clustered closely with contemporaneous Indian strains. From phylogenetic analysis, the December 2024 HRC outbreak formed a basal cluster, consistent with a single introduction followed by local transmission and subsequent spread to coastal regions. Several amino acid substitutions previously reported in association with viral replication or transmission were observed, including E2-G60D, nsP3-D4, and E1-K211T/E. The E1-A226V substitution, which has been linked to increased replication in *Aedes albopictus*, was not detected. Conclusion:

The 2024-2025 CHIKV outbreak in Kenya likely resulted from a single viral introduction in Kenya's arid north, followed by local expansion and spread to the coastal region. These results highlight the value of genomic surveillance in understanding transmission dynamics and informing public health responses in outbreak-prone regions.

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EVOLUTIONARILY DISTINCT DENGUE VIRUS 2 WITH A UNIQUE TYPE-SPECIFIC NEUTRALIZATION DETERMINANT

Sandra Vanderli Mayer[1], Evandro Reinoldo Winkelmann[1], Ana Gabriela Coello Escoto[2], Jack Dorman[2], Michael McCracken[1], Derek Cummings[3], Stephen Whitehead[2], Sonja Best[4], Dolan Patrick[2], John Aaskov[5], Gregory Gromowski[1], **Leah Katzelnick**[2]

[1]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [2]National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States, [3]Johns Hopkins University, Baltimore, MD, United States, [4]Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, Hamilton, MT, United States, [5]Retired, Brisbane, Australia

There are four recognized dengue viruses, DENV1-4, which group antigenically as serotypes. In the last 20 years, single representatives of highly divergent DENV lineages have been isolated in humans visiting rural areas on the island of Borneo. These phylogenetic intermediates have challenged the concept of four distinct DENV serotypes. Here, we describe the antigenic features of QML-22, a divergent strain that is genetically most like DENV2. We found that on average, QML-22 was better neutralized by primary DENV1, 3, and 4 sera from experimentally infected non-human primates (NHPs) than most other DENV2 viruses. However, compared to other cross-neutralized DENV2 viruses, QML-22 was less neutralized by primary DENV2 NHP sera. To evaluate if QML-22 had a distinct type-specific epitope, we depleted sera from NHPs infected with QML-22 and DENV2 reference virus S16803 with QML-22, S16803, or a mix of DENV1, 3 and 4. We found depletion with the heterotypic DENV2 had minimal effect on homotypic DENV2 neutralization, indicating distinct potently neutralizing epitopes, but affected heterotypic DENV2 neutralization, suggesting the viruses also shared a DENV2-specific epitope. To identify the targeted epitopes, we tested neutralization of diverse DENV2 strains with potent DENV2-specific antibodies. All viruses were neutralized by monoclonal antibody 2D22, which targets a quaternary epitope but is centered on envelope domain III. In contrast, antibody 3F9, which targets envelope domain I, potently neutralized other DENV2 viruses but had ~1000-fold lower neutralization against QML-22. QML-22 had numerous distinct amino acid residues in domain I compared to other DENV2 strains, including five residues in the 3F9 epitope, with only scattered point mutations elsewhere. Notably, amino acid positions in the 3F9 epitope were under less negative selective pressure than other type-specific epitopes, suggesting relaxed selection in this portion of the envelope. Collectively, this work provides insight into the diversification of the DENV serotypes and possible sites of future evolution.

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QUANTIFYING THE IMPACT OF TEMPERATURE AND TEMPERATURE FLUCTUATION ON WEST NILE VIRUS POPULATION DIVERSITY DURING MOSQUITO INFECTION

Emily A. Fitzmeyer[1], Laura C. Multini[2], David C. King[1], Courtney Murdock[2], Gregory D. Ebel[1]

[1]Colorado State University, Fort Collins, CO, United States, [2]Cornell University, Ithaca, NY, United States

RNA viruses such as SARS-CoV-2, Hepatitis C virus, and West Nile virus (WNV) exist within hosts as genetically heterogeneous mixtures of genomes, or "mutant swarms", due to error-prone mechanisms of replication. This genetic diversity is central to the evolutionary dynamics of RNA viruses. For arthropod-borne viruses like WNV, replication occurs in fluctuating ambient temperatures during infection of ectothermic mosquitoes. However, little is known about the impact of temperature and temperature fluctuation on virus population diversification and natural selection during mosquito infection and transmission. Thus, we collected bodies, legs and wings, and saliva samples from WNV-infected *Culex tarsalis* mosquitoes held at a range of stable and fluctuating temperature conditions. We then performed ultra-high-depth (~20,000X) whole WNV genome sequencing and quality-informed single nucleotide variant (SNV) calling on each sample. In WNV populations within mosquito bodies we found that missense, low frequency (<0.25) SNVs are dominant at all temperature conditions. We also observed decreasing richness (total SNVs) with increasing temperature, while selective pressure (d_N/d_S) remained stable across conditions. Ongoing analyses will quantify divergence, nucleotide diversity, and complexity in these populations. We will also incorporate additional sample types (legs and wings, saliva) - allowing us to track SNV persistence throughout mosquito infection and determine the impact of temperature and temperature fluctuation on the diversity of transmissible WNV populations.

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MAPK AND PI3K SIGNALING REGULATE ASTROCYTE NEUROINFLAMMATORY POLARIZATION IN NEUROCYSTICERCOSIS

Luz M. Toribio[1], Deborah L. Chong[2], Hector H. Garcia[1], Jon Friedland[2]

[1]Universidad Peruana Cayetano Heredia, Lima, Peru, [2]City St.George's University of London, London, United Kingdom

Astrocyte activation is a central mechanism of neuroinflammation caused by neurocysticercosis (NCC) upon *Taenia solium* larvae degeneration within the brain parenchyma. Two main astrocytes phenotypes are known (A1-like or neurotoxic and A2-like neuroprotective), yet the intracellular signaling pathways governing these responses to parasite-derived antigens remain unclear. We investigated role of MAPK and PI3K signaling in regulating astrocyte polarization in response to total *T solium* antigen extract (Tsol). Primary human astrocytes (HA) were pre-treated with specific MAPK (p38 and ERK) and PI3K inhibitors (10 μM) for 1 h prior stimulation with conditioned media from Tsol-stimulated monocytes (CoMTsol) for 4, 8, 24, 48 and 72 h. Differential secretion and gene expression of A1 (c3, ip10) and A2 (s100a, vegfa, ptx3) biomarkers was assessed ELISA and RT-qPCR, respectively. Our results show that CoMTsol stimulation induced a pro-inflammatory A1-like astrocyte phenotype characterized by increased gene expression of C3 (112.72-fold) and IP-10 (184.25-fold) compared to controls HA. Inhibition of MAPK-p38 and PI3K pathways significantly reduced expression of these biomarkers at 48 h (c3=48.3 and 6.5-fold, ip10=44.2 and 19.25-fold change compared to controls, respectively), while ip-10 secretion dropped at 72 h (from 4035.55 to 877.5 pg/ml) indicating that these pathways are required for antigen-induced astrocyte activation. In contrast, inhibition was associated with a relative increase in vegfa gene expression (8.70 and 4.3-fold change for MAPK-p38 and PI3K inhibition, respectively), suggesting a shift toward a reparative phenotype. Inhibition of the MAPK-ERK signaling pathway did not show significant effect at any timepoint (p>0.61). These findings identify PI3K and MAPK-p38 signaling pathways as key regulators of astrocyte polarization to an inflammatory state, underscoring the intracellular mechanisms of NCC pathology to propose novel therapeutic strategies that may modulate neuroinflammation in NCC.

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TAENIA SOLIUM LARVAL STAGE PROTEINS INDUCE NEURONAL DAMAGE: IDENTIFICATION OF NEUROTOXIC FRACTIONS AND PROTEOMIC PROFILE

José Octavio Zapata More[1], Nancy Chile Andrade[1], Gino Castillo Vilca[1], Manuela Verastegui Pimentel[1], Robert H. Gilman[2]

[1]Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Johns Hopkins University, Baltimore, Peru

Neurocysticercosis (NCC), caused by the larval stage of *Taenia solium*, is a major neurological disease in endemic regions like Peru associated with seizures and cognitive deficits. While damage is traditionally attributed to the host immune response, recent evidence of axonal spheroids in areas with minimal inflammation suggests a direct neurotoxic effect

through excretory/secretory (ES) proteins. These spheroids represent early neurodegeneration, reflecting impaired axonal transport and protein aggregation. This study determined the role of ES proteins and their neurotoxic fractions in inducing neuronal damage while characterizing the proteomic profile. ES proteins from porcine cysticerci were separated into 22 fractions via anion-exchange chromatography and applied to primary rat hippocampal cultures at 20 µg/mL for 10 hours. Axonal damage was quantified via indirect immunofluorescence for beta-III-tubulin to calculate the spheroid-per-neuron ratio. The most neurotoxic fractions (F8 and F20) underwent nano LC-MS/MS analysis to identify effectors. Results revealed that neurotoxicity is concentrated in specific subpopulations rather than the entire secretome. Specifically, fractions F2, F8, F16, F17, F20, and F21 exclusively induced axonal spheroids, with F20 and F21 causing the most significant damage, while fractions F5, F6, F13, and F19 induced axonal fragmentation. Proteomic analysis identified 12 shared proteins between F8 and F20, including nardilysin (linked to autophagy dysregulation), calreticulin (calcium homeostasis), poly U-specific endoribonuclease, puromycin-sensitive aminopeptidase, and peroxidasin. These findings demonstrate that T. solium secretes a coordinated arsenal of neurotoxic effectors acting synergistically to collapse the neuronal cytoskeleton. This study shifts the NCC paradigm by suggesting that the parasite actively hijacks vital processes, such as functional autophagy and axonal structural stability, leading to irreversible neurodegeneration. Identifying these 12 proteins provides new molecular targets to mitigate neurological sequelae in NCC patients.

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DIFFERENTIAL PROPORTIONS OF CD4 AND CD8 SUBSETS BETWEEN NEUROCYSTICERCOSIS PHENOTYPES AND A BYSTANDER EFFECT DURING VIABLE INFECTION

Sabrina Hammond[1], Thomas B. Nutman[2], Elise OConnell[3]

[1]National Institutes of Health, Bethesda, MD, United States, [2]National Institutes of Health, Chevy Chase, MD, United States, [3]NIH/NIAID, Bethesda, MD, United States

Neurocysticercosis (NCC) is a parasitic disease caused by *Taenia solium* infection of the central nervous system. Inflammation contributes to the spectrum of symptoms across phenotypes. Previously we have described the localized CSF inflammatory milieu in subarachnoid NCC (SANCC), including that relative levels of IL-10 are proportional to time to parasite CSF clearance. We have also shown the major source of local IL-10 in SANCC is $CD4^+CD127^-FoxP3^-$ cells. To begin to understand immunologic differences that may predispose patients to the development of SANCC compared to parenchymal NCC (PNCC), we sought to compare baseline peripheral blood mononuclear cell (PBMC) populations and differential antigenic responses of those with SANCC, PNCC, and healthy controls (HC). To provide insight into the nature of the systemic T cell response in NCC, we used a T cell focused multiparameter flow cytometry panel to assess the response of PBMCs in 10 SANCC subjects, 10 PNCC subjects, and 10 HC subjects at baseline and following exposure to optimal concentrations of T. crassiceps soluble antigen (TcAg), CEFX (a multi viral peptide pool), soluble *Candida albicans* extract, and Staphylococcus enterococcus B (SEB) superantigen. At baseline, NCC infected patients had a significantly expanded population of $CD4^+CCR7^-CD45RA^+$ TEMRA cells compared to HC (p=0.042). Within the NCC patients, those with SANCC had markedly elevated numbers of activated $CD4^+CCR6^+CCR4^+CD25^+$ cells (p=0.0045), and those with PNCC had an expansion of $CD8^+CCR7^-CD45RA^+$ TEMRA cells (p=0.024). While the response to SEB was equivalent across all groups, upregulation of CD25 and CD69 in response to control antigens in those with NCC (both SANCC and PNCC) were diminished compared to HC. Interestingly, in 6 NCC patients' PBMCs taken following definitive cure, there was evidence of reversal of the bystander antigen-specific hyporesponsiveness in the $CD4^+$ compartment in response to control antigens. These data suggest that NCC is associated with a reversible, hyporesponsive immune state induced by active parasite infection that may be driven by immunomodulatory secreted parasite molecules.

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IMMUNE RESPONSES ASSOCIATED WITH PARTIAL PROTECTION INDUCED BY REPEATED *NECATOR AMERICANUS* LARVAL INFECTION AND TREATMENT

Friederike Sonnet

Weill Cornell Medicine, New York, NY, United States

With an estimated 450 million chronically infected people, hookworm infection remains a pervasive public health issue, particularly in low- and middle-income countries. Mass drug administration has not broken transmission cycles, therefore an effective vaccine is needed. However, it is not clear whether immunity to this parasite can develop. Repeatedly exposed individuals in areas endemic for hookworm infection appear to not develop a protective immunity. This has largely been attributed to the immune modulation and suppression by the parasite. Yet, repeated infections in controlled human hookworm infection models in naïve volunteers, have indicated that resistance to infection can develop. This provides an opportunity to examine immune responses associated with protection from hookworm infection. Peripheral blood mononuclear cells were collected in a placebo-controlled trial, where the repeated short-term exposure of naïve volunteers to hookworm larvae (L3) was shown to reduce worm burden upon challenge. We performed comprehensive high-dimensional immune profiling on these cells using mass cytometry and spectral flow cytometry. The repeated exposure to L3 resulted in a type 2 polarization and a short expansion of CD161+ regulatory T cells with a T helper 2-like phenotype. Upon challenge, we compared the immune profiles in the susceptible volunteers versus those who controlled their infection. Here, a cluster of myeloid antigen presenting cells, CRTH2+ CD4+ effector memory cells, and high frequencies of CXCR3+ T helper 1-like regulatory T cells were associated with protection. In addition, volunteers who controlled the infection, had high antigen-specific IL-9, IFN-γ, and TNF responses during the immunization phase and experienced a severe skin reaction at larval penetration sites. Overall, we observe multifaceted and heterogeneous type 2 responses associated with hookworm infection. We unraveled specialized subpopulations of cells that were linked to protection, highlighting the value of high-dimensional immunology tools, which can guide vaccine development.

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BASOPHIL METABOLIC PROFILING SUGGESTS NONDEGRANULATORY IGE-MEDIATED ACTIVATION TO *TRICHURIS TRICHIURA*

Harris R. Droghini[1], George Sellers[1], P'ng Loke[1], Thomas B. Nutman[2]

[1]National Institutes of Health, Bethesda, MD, United States, [2]National Institutes of Health, Chevy Chase, MD, United States

Basophils are the only circulating cell that degranulate and release histamine in an IgE-dependent manner. Basophil depletion in mice is associated with impaired ability to clear *Trichuris muris* infection. *Trichuris trichiura* infects hundreds of millions of people and is associated with increased levels of total and *Trichuris*-specific IgE, the latter correlating negatively with infection burden. However, the role of basophils in human trichuriasis is not known. 25 patients with proven trichuriasis and 10 uninfected controls were profiled for serum *Trichuris*-specific immunoglobulins by ELISA; a subset (n=8) underwent basophil activation testing to anti-human IgE and *T. muris* excretory/secretory (Tm ES) antigen with an intracellular metabolic panel of flow cytometric-compatible antibodies to evaluate glycolysis (GLUT1), the pentose-phosphate pathway (G6PD), the Krebs cycle (ATP5A and SDHA), and fatty acid metabolism (CPT1A). There was an expected heterogeneity of specific IgG, IgG4, and IgE responses, with 8 patients producing an elevated quantity of Trichuris-specific IgE. We saw a significant increase in median fluorescence intensity (MFI) in ATP5A (41.5%) and CPT1A (12.4%) with anti-IgE stimulation, a finding suggesting key contributions of fatty acid oxidation and the Krebs cycle in basophil activation. Preliminary analysis of mass spectrometry data from blood bank samples orthogonally validates IgE-induced metabolic alteration of basophils. Upon stimulation with Tm ES,

CPT1A and ATP5A were elevated independent of level of *Trichuris*-specific IgE and in absence of CD63 upregulation (a marker of degranulation), possibly indicating basophil activation not resulting in histamine release. Testing 5 *Trichuris*-naïve controls showed decrease of ATP5A (20.6%) and SDHA (25.8%), suggesting that a component of the antigen directly inhibits basophil Krebs cycle. Taken together, IgE-mediated basophil nondegranulatory responses may overcome direct immunoregulatory activity of *Trichuris* antigen to combat infection. This hypothesis is being investigated in an ongoing *T. trichiura* controlled human infection model.

6500

PROTEOMIC IDENTIFICATION OF NOVEL DIAGNOSTIC TARGETS FOR ONCHOCERCIASIS IN EXTRACELLULAR VESICLES ISOLATED FROM PLASMA

Lucia S. Di Maggio[1], Sarah E. Greene[1], Amy Rush[1], Abakar Gankpala[1], Kurt C. Curtis[1], Kerstin Fischer[1], Bruce A. Rosa[1], Byoung-Kyu Cho[2], Young Ah Goo[2], Makedonka Mitreva[1], Peter Fischer[1]

[1]Infectious Diseases Division, Department of Medicine, Washington University School of Medicine, Saint Louis, MO, United States, [2]Mass Spectrometry Technology Access Center at McDonnell Genome Institute, Washington University School of Medicine, St. Louis, Missouri, United States of America, Saint Louis, MO, United States

Onchocerciasis (river blindness) is caused by the filarial nematode *Onchocerca volvulus* and remains a major public health challenge for millions of people in sub-Saharan Africa. Global elimination programs led by the World Health Organization (WHO) have emphasized the need for more sensitive diagnostics, improved therapeutics and innovative treatment regiments. Helminth-derived extracellular vesicles (EVs) are important mediators of host–pathogen communication, as well as promising targets for parasite detection. We analyzed EVs from plasma of 30 *O. volvulus*–infected patients with various microfilaria (Mf) densities from Ghana, Uganda, and Liberia. EVs were isolated using the ME kit (Vn96 synthetic peptide affinity–based method), followed by protein digestion and data-independent acquisition (DIA) mass spectrometry. Resulting spectra were searched with Spectronaut against *O. volvulus* and human protein databases. Thirty-seven parasite proteins were identified in total: 4 in samples from Ghana, 13 in samples from Uganda, and 23 in samples from Liberia. Among these proteins, only OVOC6777 was detected in samples from all three locations and in 77% of the patients. OVOC6777 is a nematode-specific protein with sequence similarity to intermediate filament rod-domain proteins from other nematodes. Another promising target was OVOC8736, an *O. volvulus*–specific protein of unknown function, that was present in more than half of the Ghanaian and Ugandan samples (12/20), thus will be investigated further in future studies. We generated polyclonal mouse antibodies against OVOC6777 for immunolocalization. OVOC6777 was found in viable adult female worms mostly in the uterus wall, developing Mf, muscle tissue, and the lateral cords. The antibody labeled also muscles and lateral cords in adult males but did not stain any dead worms or surrounding nodule tissues. These findings justify further investigating the potential of OVOC6777 as a useful biomarker for an onchocerciasis antigen detection test.

6501

HOST GENETICS AND THE GUT MICROBIOTA SHAPE RESISTANCE TO *ASCARIS* INFECTION IN MICE

Fabricio Marcus Silva Oliveira[1], Aline Sardinha-Silva[2], Juan Camilo Sanchez-Arcila[3], Ejae Bewaji[2], Hongyu Wu[4], Eric Dang[2], Pedro Henrique Gazzinelli-Guimaraes[1]

[1]The George Washington University, Washington, DC, United States, [2]National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States, [3]School of Natural Sciences, University of California, Merced, CA, United States, [4]National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, DC, United States

Ascaris spp. are the most prevalent helminth parasites worldwide and induce a robust Type 2 inflammatory response as a result of their transient larval migration through host tissues. However, the contributions of host genetics and the gut microbiota to parasite burden and immune responses to *Ascaris* remain poorly defined. We investigated *Ascaris* infection and the associated pathology across several genetically diverse mice. CAST/EiJ and WSB/EiJ mice, as well as A/J mice, exhibited a marked reduction in parasite burden compared with the highly susceptible C57BL/6J strain. At the peak of larva migration in the lungs (day 8), resistance in CAST/EiJ mice was associated with an increase in the infiltration of SiglecF$^+$ eosinophils and elevated production of GM-CSF, IL-5, IL-13, and CCL-11. Additionally, CAST/EiJ mice developed pronounced lung pathology with granuloma formation, which was absent in C57BL/6J mice. Eosinophil depletion in CAST/EiJ mice via anti-IL-5 reduced lung immunopathology without affecting resistance, indicating that resistance is independent of lung-stage clearance. Kinetic studies confirmed that CAST/EiJ mice exhibited reduced larval burdens as early as 2 days post-infection, consistent with early resistance during the gastrointestinal phase. To determine whether resistance was genetically dominant, we performed reciprocal crosses between CAST/EiJ and C57BL/6J mice. CAST/EiJ male × C57BL/6J female F1 progeny were highly susceptible, whereas CAST/EiJ female × C57BL/6J male F1 progeny were resistant, suggesting a potential role for vertical microbiome transmission. Consistently, germ-free C57BL/6 mice displayed marked protection compared with specific pathogen-free controls. Furthermore, fecal microbiota transplantation experiments in germ-free C57BL/6 mice, using either CAST/EiJ or C57BL/6J cecal contents, demonstrated that microbiota derived from CAST/EiJ mice conferred greater protection than that from C57BL/6J mice. Together, these findings highlight a critical interplay between host genetics and the gut microbiota in shaping parasitism and tissue pathology during *Ascaris* infection.

6502

RESPIQUESTAB: AN EVIDENCE-BASED ELECTRONIC CLINICAL DECISION SUPPORT TOOL TO IMPROVE DIAGNOSIS AND MANAGEMENT OF LOWER RESPIRATORY TRACT INFECTIONS IN A LOW- OR MIDDLE-INCOME COUNTRY SETTING

Armstrong Obale[1], Stefany Olague[2], Eric Laber[3], John Gallis[3], Maria Iglesias-Ussel[4], Dhammika Palagasinghe[5], Jayani Gamage[6], Bhagya Senadheera[6], Sewwandi Vasana[6], Champica Bodinayake[5], Ajith Nagahawatte[7], WMDGB Wijayaratne[7], Ruvini Kurukulasooriya[6], James Ngocho[8], Truls Østbye[9], Melissa Watt[10], Evan Myers[4], Christopher Woods[4], Susanna Naggie[4], Hrishikesh Chakraborty[3], Avi Kenny[3], P.D.W.D De Zoysa[5], Gayani Tillekeratne[4]

[1]Hubert-Yeargan Center for Global Health, Duke Global Health Institute, Duke University, Durham, NC, United States, [2]Duke Clinical Research Institute, Duke University School of Medicine, Durham, NC, United States, [3]Department of Biostatistics and Bioinformatics, Duke University, Durham, NC, United States, [4]Department of Medicine, Duke University School of Medicine, Durham, NC, United States, [5]Department of Medicine, Faculty of Medicine, University of Ruhuna, Galle, Sri Lanka, [6]Duke-Ruhuna Collaborative Research Centre, Faculty of Medicine, University of Ruhuna, Galle, Sri Lanka, [7]Department of Microbiology, Faculty of Medicine, University of Ruhuna, Galle, Sri Lanka, [8]Kilimanjaro Christian Medical Centre, Moshi, United Republic of Tanzania, [9]Department of Family Medicine and Community Health, Duke University, Durham, NC, United States, [10]Department of Population Health Sciences, School of Medicine, University of Utah, Salt Lake City, UT, United States

Lower respiratory tract infections (LRTI) account for a large burden of disease globally. Etiology of LRTI is difficult to diagnose based on clinical parameters, leading to inappropriate antimicrobial use and downstream antimicrobial resistance. We present RespiQuestAB, an electronic clinical decision support tool (eCDST), developed with the goal of improving the diagnosis of LRTI etiology and appropriate antimicrobial prescription in Sri Lanka. We trained a machine learning (ML) algorithm that integrates

multiple low-cost data inputs: 1) real-time surveillance data for influenza in Sri Lanka from the World Health Organization FluNET data; 2) patient-level clinical variables associated with etiologic class of infection from a dataset of 521 patients with LRTI we previously enrolled in Sri Lanka; 3) point-of-care (POC) pathogen tests for influenza, SARS-CoV-2, and *Streptococcus pneumoniae;* and 4) the POC biomarker test for procalcitonin. The algorithm is decision tree-based and was translated into an electronic application, RespiQuestAB, that physicians could use on their smart phones.We conducted interviews and focus groups with local physicians pre- and post-development to refine the application. RespiQuestAB allows for the input of up to 12 clinical variables, such as fever, shortness of breath, and white blood cell count. The application returns the probabilities of the following diagnoses: bacterial, influenza, other viral infection, non-infectious, and indeterminate. Treatment with antibacterials and oseltamivir (for influenza) is recommended or discouraged based on most likely diagnosis. Incorporated within the app are links to treatment guidelines for LRTI from the Sri Lankan College of Microbiologists. RespiQuestAB has the potential to improve the diagnosis and management of LRTI. A stepped-wedge, cluster-randomized clinical trial is currently underway at three public hospitals in Sri Lanka to determine the impact of RespiQuestAB on clinical outcomes and antimicrobial use.

6503

EFFECTIVENESS OF PCV10 AND PCV13 AGAINST INVASIVE PNEUMOCOCCAL DISEASE AMONG CHILDREN IN VIETNAM PRIOR TO NATIONAL INTRODUCTION: A MATCHED CASE-CONTROL STUDY

Hieu Cong Truong[1], Thuong Vu Nguyen[1], Niko Speybroeck[2]

[1]Pasteur Institute in Ho Chi Minh City, Ho Chi Minh, Vietnam, [2]Institute of Health and Society, Bruxelles, Belgium

Evidence on pneumococcal conjugate vaccine (PCV) effectiveness in Vietnam is limited, despite the availability of PCV10 and PCV13 in the private sector. We evaluated the effectiveness of PCVs against invasive pneumococcal disease (IPD) among children prior to national introduction. We conducted a matched case-control study from February 2022 to January 2025 in southern Vietnam. Cases were children aged 2-59 months hospitalized with culture-confirmed IPD at three tertiary pediatric hospitals, with four age- and neighborhood-matched community controls per case. Vaccine effectiveness (VE) was estimated using conditional logistic regression. We enrolled 72 IPD cases and 288 controls; 37.5% of cases had received ≥1 PCV dose. Most cases presented with meningitis (36.1%) or pneumonia-associated sepsis (30.6%). Serotype 19A was predominant (26.4%), and 77.8% of cases were caused by PCV13 serotypes. Adjusted VE against vaccine-type IPD was high for both PCV10 and PCV13 (≥86% for ≥1 dose). Against all-serotype IPD, VE was 65.1% (95% CI: 21.8-84.4) for ≥1 PCV10 dose and 84.4% (95% CI: 20.0-97.0) for ≥1 PCV13 dose. No significant protection was observed against non-PCV10-type IPD, and VE declined with time since vaccination. PCV10 and PCV13 provide substantial protection against vaccine-type and overall IPD among young children in Vietnam. The predominance of serotype 19A and evidence of waning protection support the use of higher-valency vaccines and booster schedules, providing critical evidence to inform national PCV introduction in a high-burden setting.

6504

WASTEWATER DETECTIONS OF *BORDETELLA PERTUSSIS* AND *MYCOBACTERIUM TUBERCULOSIS* NUCLEIC ACIDS IN ACTIVE DISEASE OUTBREAK SITES IN THE USA

Abby Paulos[1], Alessandro Zulli[2], Dorothea Duong[3], Bridgette Shelden[3], Bradley White[4], Devin North[1], Alexandria Boehm[5], Marlene Wolfe[1]

[1]Emory University, Atlanta, GA, United States, [2]Stanford University, Palo Alto, CA, United States, [3]Verily Life Sciences, San Francisco, CA, United States, [4]Google Inc, Mountain View, CA, United States, [5]Stanford, CA, United States

Cases of bacterial respiratory pathogens like *Mycobacterium tuberculosis* and *Bordetella pertussis* have increased since the COVID-19 pandemic, yet clinical surveillance of both suffers from underreporting and delayed diagnoses. Wastewater monitoring is a valuable public health surveillance tool that can help fill gaps in clinical data, yet has rarely been applied to respiratory bacterial pathogens despite evidence of bacterial shedding into excretion types that enter wastewater. In this study, we investigated the possibility for wastewater monitoring of two bacterial respiratory diseases, tuberculosis and pertussis and present two case studies of wastewater monitoring for *M. tuberculosis* and *B. pertussis*. We retrospectively measured concentrations of these pathogens in wastewater samples collected longitudinally from communities with and without known outbreaks of these diseases. We designed and validated a novel *B. pertussis*-specific assay for the NAD(P) gene, yet *B. pertussis* nucleic acids were rarely detected in wastewater during an identified outbreak. We used a highly specific, established assay for *M. tuberculosis* nucleic acids, and found low concentrations of the marker in wastewater that were lag-correlated with clinical incidence rates 5 weeks later. Findings support the potential of wastewater monitoring for *M. tuberculosis* and *B. pertussis* to enable identification of communities with outbreaks of tuberculosis and pertussis and provide early warning for tuberculosis.

6505

TRUE INCIDENCE OF HUMAN METAPNEUMOVIRUS HOSPITALIZATIONS ACROSS VARYING CLIMATE ZONES IN AUSTRALIAN CHILDREN

Belaynew Wasie Taye[1], Mohinder Sarna[2], Damien Foo[2], Avram Levy[3], Cara Minney-Smith[4], Peter Richmond[5], Christopher Blyth[6], Hannah Moore[2]

[1]University of Western Australia, Nedlands, Australia, [2]The Kids Research Institute Australia Australia, Perth, Australia, [3]University of Western Australia, Perth, Australia, [4]PathWest Laboratory Medicine, Department of Health, Perth, Australia, [5]Perth Children's Hospital, Perth, Australia, [6]University of Western Australia, Nedlands, Ethiopia

Climatic variables such as temperature, length of sunshine and wind are known to derive the pattern of human metapneumovirus(hMPV)-related acute respiratory infections. However, up to date information is not available in Western Australia (WA). This study aimed to develop and validate a prediction model to estimate the true burden of hMPV hospitalizations across tropical, subtropical, and temperate climates in WA children. A population-based cohort study of children aged less than 15 years born in WA was conducted between 01 January 2010 and 30 April 2024. We used linked perinatal, hospitalization and routine microbiological testing data from the Respiratory Infections Linked Data platform. We developed and validated prediction models (logistic regression, random forest, and Extreme Gradient Booting) using machine learning algorithms. Variables were initially selected using LASSO-penalised logistic regression. Predicted probabilities were estimated using Extreme Gradient Boosting model. We compared laboratory-confirmed hospitalisation rates with model-predicted rates and determined the under-ascertainment fractions. Our cohort included 668,341 hospital admissions (308,051 children), of whom 56,829 were hMPV-tested (4.1% positive). The true incidence rate of hMPV was higher in the tropical (5.9 per 1,000 child-years for infants in northern tropical regions), and subtropical desert (6.5 per 1,000 child-years for infants in central desert) regions compared with the temperate areas (1.6 cases

per 1,000 child-years for infants in Southern temperate regions). Routine microbiological testing underestimated the true burden of hMPV by 30 - 50%. In conclusion,the burden of hMPV varies significantly across climate regions informing the need for localized decisions in prioritising for testing and preventive measures. High under-ascertainment in children highlights the need to increase testing to better identify children with hMPV for timely treatment, as well as prevention of healthcare-associated and community transmission.

6506

CLINICAL PREDICTION MODEL FOR VIRAL ETIOLOGY AMONG YOUNG CHILDREN WITH PNEUMONIA IN THE GAMBIA, MALI, AND SOUTH AFRICA

James Samwel Ngocho[1], Edwin Shewiyo[1], Blandina Theophil Mmbaga[2], Benjamin J. Brintz[3], Matthew Rubach[4], Daniel T. Leung[5]

[1]KCMC University, Moshi, United Republic of Tanzania, [2]Kilimanjaro Clinical Research Institute, Kilimanjaro, United Republic of Tanzania, [3]University of Utah, Salt Lake City, UT, United States, [4]Duke University, Durham, NC, United States, [5]Univ of Utah School of Medicine, Salt Lake City, UT, United States

A significant attributable risk factor for antimicrobial resistance is unnecessary administration of antibacterials when there is not a proper indication. Though research has shown that viruses cause most of the pneumonia among children ≤5 years of age (≤5-y) in low- and middle-income countries, management guidelines still favour empiric antibacterials. Clinicians need practical tools to help them differentiate bacterial vs. viral causes of pneumonia Using data from the Pneumonia Etiology Research for Child Health (PERCH) study, we developed and internally validated a clinical prediction model to identify viral pneumonia in children ≤5-y. Viral etiology was defined as a posterior probability of ≥0.9 based on PERCH integrated etiology estimates. We used a random forest algorithm for feature selection and developed predictive models, including random forest and LASSO logistic regression, evaluated through 10-fold stratified cross-validation. Our study included 2,072 children with pneumonia from Mali, The Gambia, and South Africa, of whom 683 (33.0%) were classified as viral pneumonia. Six routinely available clinical variables emerged as significant predictors in the model: age, heart rate, duration of illness, body temperature, ambient temperature, and respiratory rate. The final model (random forest) achieved an area under the receiver operating characteristic curve of 0.70, with an accuracy of 0.69, sensitivity of 0.34, specificity of 0.86, positive predictive value of 0.55, and negative predictive value of 0.73.This parsimonious clinical prediction model derived from routinely collected clinical variables achieve moderate discrimination for viral pneumonia in children ≤5-y. The model's high specificity suggests utility for ruling in viral pneumonia. External validation in diverse settings is warranted to assess model generalizability, and clinical utility prior to broader implementation. Future research may explore integrating patient biomarkers into this clinical prediction model to further improve its performance.

6507

DISCOVERING *MYCOBACTERIUM TUBERCULOSIS*-SPECIFIC MONOCLONAL ANTIBODIES FROM TUBERCULOSIS SURVIVORS

Shi-Hsia Hwa[1], Peter M. Finin[2], Xiang Yu[2], Shunsuke Sakai[3], Ashley Jacobs[4], Ming Zhao[1], Iyadh Douagi[2], Daniel L. Barber[2], Clifton E. Barry III[2], Robert J. Wilkinson[5], Joshua Tan[6]

[1]National Institute of Allergy and Infectious Diseases, Rockville, MD, United States, [2]National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States, [3]Institute for Vaccine Research and Development, Hokkaido University, Sapporo, Japan, [4]University of Cape Town, Cape Town, South Africa, [5]Institute of Infectious Disease and Molecular Medicine, University of Cape Town, Cape Town, South Africa, [6]National Institutes of Health, Rockville, MD, United States

Tuberculosis (TB) has overtaken Covid-19 to become the deadliest infectious disease in the world again. Antimicrobial resistance is a serious threat to our ability to cure and control TB using antibiotics, prompting the need for other therapeutics. Furthermore, BCG has low vaccine efficacy against adult pulmonary TB and the current vaccine development pipeline is sparse. Despite evidence from animal and human studies that B cells and antibodies may have a role in protection against TB, the antibody response to *Mycobacterium tuberculosis* (Mtb) is still not well understood. Most previous studies on human Mtb antibodies have yielded a relatively small number of monoclonal antibodies (mAbs) and focused on a limited set of known antigens, compared to the huge variety of macromolecules present on the bacterial surface. We have developed a method for unbiased, antigen-agnostic discovery of antibodies reactive with the surface of Mtb, using whole inactivated bacilli as a probe in a multi-step screening process to first down-select B cell cultures producing anti-Mtb IgA or IgG, then using an optofluidic platform to identify and isolate positive single cells. This pipeline was used to isolate Mtb-specific B cells from peripheral blood of TB survivor participants from the PredictTB treatment-shortening trial. We recovered over 60 monoclonal antibodies with natural heavy and light chain pairings, and confirmed binding to whole bacilli. Among these TB survivor-derived antibodies, several mAbs were identified that bind known Mtb protein antigens such as Antigen 85A and PstS1, or lipoglycan antigens such as lipoarabinomannan. Some of these bind as strongly or better compared to previously published mAbs. The targets of the other strong whole-bacteria-binding mAbs are in the process of being identified by immunoassay and proteomic methods. We are also testing these mAbs for functional protection *in vivo* in mice. This project could potentially yield antibody immunotherapies for TB to be used as an adjunct to antibiotics, as well as reveal novel targets for next-generation vaccines.

6508

OUTPATIENT MANAGEMENT OF CHILDREN WITH CHEST INDRAWING PNEUMONIA IN PRIMARY HEALTHCARE SETTINGS IN PUNJAB, PAKISTAN- A PROSPECTIVE COHORT STUDY

Zamir Hussain Suhag

Trust for Vaccines & Immunizations, Karachi, Pakistan

Pneumonia remains a leading cause of under-five mortality in Pakistan. The government of Pakistan revised the national Integrated Management of Childhood Illness (IMCI) protocol in 2019, recommending outpatient treatment of pneumonia, characterized by fast breathing and/or chest indrawing, with oral amoxicillin in children aged 2-59 months. This prospective observational cohort study was conducted at five PHC facilities in the peri-urban areas of Lahore and evaluated caregiver-reported outcomes in children presenting with chest indrawing pneumonia at primary healthcare (PHC) facilities of Lahore. Children aged 2-59 months with chest indrawing pneumonia were enrolled and followed up on day 15 post-enrolment. The primary outcome was the case-fatality rate (CFR). Secondary outcomes included antibiotic use, treatment adherence, and hospitalization. We conducted verbal autopsies for children who died during the follow-up period. We enrolled 360 children, and day 15 follow-up was completed in 356 (98.8%). The CFR for chest indrawing pneumonia was 0.6% (2/356; 95% CI: 0.1-2.2%). At follow-up, caregivers reported 336/356 (94.4%) as cured, while 12/356 (3.4%) not improved or worsened. Healthcare workers prescribed oral amoxicillin to 192/356 (53.9%) children, of whom 160/192 (83.3%) adhered to the specified duration of four or more days. On day 15 follow-up, 26/356 (7.3%) caregivers reported switch to other antibiotics during treatment. Among these, 7/26 (27%) were hospitalised and received injectable antibiotics, 5/26 (19.2%) were managed as outpatients with injectable antibiotics, and 14/26 (53.8%) were treated with different oral antibiotics on an outpatient basis. The low CFR and high cure rate among children treated on an outpatient basis with oral antibiotics at the PHC settings supports the IMCI protocol. Management of chest indrawing pneumonia on an outpatient basis at the primary care level is feasible, with the potential to reduce unnecessary hospital referrals, reduce antimicrobial resistance, lower healthcare costs, and decrease mortality, particularly in resource-constrained settings.

6509

IMMUNE PROFILING OF NK CELLS, UNCONVENTIONAL T CELLS, AND B CELLS IN TUBERCULOSIS REVEALS BIOMARKERS FOR DIAGNOSIS AND TREATMENT RESPONSE

ISAAC ACHEAMPONG[1], Edwin F. Laing[1], Michael Frimpong[1], Difery Minadzi[2], Ernest Adankwah[1], Dorcas Ohui Owusu[1], Linda Batsa Debrah[1], Alexander Yaw Debrah[1], Richard Odame Phillips[2], Marc Jacobsen[3]

[1]Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, [2]Kumasi Centre for Collaborative Research in Tropical Medicine, (KCCR), Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, [3]Heinrich-Heine University, Duesseldorf, Germany

The immunopathogenesis of tuberculosis (TB) reflects complex interactions between innate and adaptive immune responses. In TB-endemic settings such as Ghana, interpretation of immunological diagnostics is complicated by prior BCG vaccination. This study characterised Natural Killer (NK) cells, unconventional T-cells, and B-cell subsets to identify immune signatures with potential diagnostic and treatment-monitoring value. A case-control design was used, involving active TB patients (n=89) and healthy controls (n=46), with a longitudinal follow-up of 51 TB patients over 16 weeks of anti-TB treatment. NK cell subsets ($CD38^{+/-}$), MAIT cells ($CD8^{+}V\alpha7.2^{+}CD161^{+}$), γδ T-cells, and B-cell subsets ($CD19^{+}CD27^{hi/lo}$) were analysed by flow cytometry. Active TB was associated with an expansion of activated $CD3^{-}CD56^{+}CD38^{+}$ NK cells and a reduction in $CD3^{-}CD56^{+}CD38^{-}$ NK cells ($p<0.0001$), both showing good discriminatory performance (ROC=0.77 and 0.81, respectively). Within the adaptive compartment, total B-cells ($CD3^{-}CD19^{+}$; $p=0.0001$) and $CD27^{hi}$ B-cells ($p<0.0001$) were significantly reduced in TB patients. Unconventional T-cell populations exhibited severity-dependent changes: $CD8^{+}$γδ T-cells ($p=0.0042$) and $CD4^{+}CD161^{+}$ T-cells ($p=0.0163$) were elevated mainly in paucibacillary disease, indicating a stronger innate-like response in less advanced infection. In contrast, MAIT cells were consistently reduced in active TB ($p<0.0001$). Importantly, NK cell and B-cell signatures remained reliable irrespective of BCG vaccination status. During treatment, $CD38^{+}$ NK cells declined significantly toward baseline by week 16, whereas B-cell subsets showed no significant change. Overall, TB is associated with distinct alterations in innate and B-cell compartments that are independent of BCG-related effects. The pattern of CD38 expression on NK cells, in particular, shows potential as both a diagnostic marker and an indicator of treatment response in endemic settings.

6510

TARGETED SEQUENCING OF KEY *MYCOBACTERIUM TUBERCULOSIS* RESISTANCE GENES TO DETERMINE GENOTYPIC RESISTANCE PROFILES AMONG CLINICAL ISOLATES WITH DISCORDANT PHENOTYPIC DRUG SUSCEPTIBILITY RESULTS

Gustiani Salim[1], Deni Pepy Butar Butar[1], Adhella Menur Naysilla[1], Melinda Setiyaningrum[1], Erlina Burhan[2], Lidya Chaidir[3], Robert Sinto[4], Dewi Lokida[5], **Aaron T Neal**[6]

[1]INA-RESPOND, Jakarta, Indonesia, [2]Universitas Indonesia, Persahabatan Hospital, Jakarta, Indonesia, [3]Universitas Padjadjaran, Bandung, Indonesia, [4]Cipto Mangunkusumo Hospital, INA-RESPOND, Jakarta, Indonesia, [5]Tangerang District Hospital, INA-RESPOND, Jakarta, Indonesia, [6]National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States

Indonesia has the second-highest burden of tuberculosis (TB) and the second-largest gap between estimated incidence and reported cases. Of the estimated 24,637 multidrug-resistant/rifampicin-resistant (MDR/RR) TB cases in 2022, only 50.7% were notified, with treatment success ranging from 45-68%. Rapid and accurate drug-resistance testing is crucial for diagnosis and regimen design, though the reference standard of phenotypic drug-susceptibility testing (pDST) often produces inconsistent results. We performed targeted Sanger sequencing of *Mycobacterium tuberculosis* culture isolates to resolve discordant pDST results. Isolates were obtained from the TRIPOD drug-resistant TB study, which enrolled 490 adults with presumptive TB from seven provincial hospitals in Indonesia during 2017-2020. Xpert MTB/RIF, culture, and pDST were performed from the same sputum sample prior to TB treatment, with many participants having additional independent pDST results from standard of care procedures. After TRIPOD study completion, isolates with discordant results were thawed from -80°C and sequenced for rpoB (RIF); *inhA*, *fabG1*, *katG* (INH); *embB* (EMB); *gyrA*, *gyrB* (FQ); and *rpsL*, *rrs, eis* (aminoglycosides). Mutations were characterized using the WHO 2021 catalogue. pDST discordance was identified in 31/137 (22.6%) Xpert MTB/RIF resistant participants, most frequently in EMB (48.4%) and INH (35.5%). Sequencing identified key resistance mutations in *rpoB* (93.5%; predominantly S450L), *katG* (41.9%; S315T), *embB* (19.4%; M306V), *gyrA* (12.9%; A90V), and *rpsL* (3.2%; K43R). Overall, 64.5% of isolates showed concordance between phenotypic and genotypic classification. Sequencing reclassified five rifampicin-sensitive isolates as MDR/RR-TB and two ofloxacin-sensitive isolates as pre-XDR-TB. Discordance in pDST may reflect sample variability or technical factors, but targeted Sanger sequencing can overcome these by providing complementary genotype data, improving the detection of drug resistance. Future studies should assess direct applications to clinical samples, inclusion of newer drugs, and impact on treatment outcomes.

6511

MOLECULAR CONFIRMATION AND *IN SILICO* STRUCTURAL CHARACTERIZATION OF RIBONUCLEOTIDE REDUCTASE SMALL SUBUNIT IN *ONCHOCERCA VOLVULUS* AS A TARGET FOR ANTIMETABOLITE DRUG REPURPOSING

Prince-Charles Kudzordzi[1], John Asiedu Larbi[2], Anabel Acheampong[3], Prince Nyarko[4], Kenneth Bentum Otabil[5]

[1]Consortium for Neglected Tropical Diseases and One Health, University of Energy and Natural Resources, Sunyani, Ghana, [2]Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, [3]Consortium for Neglected Tropical Disease and One Health, Sunyani, Ghana, [4]The Consortium for Neglected Tropical Diseases and One Health (NeTroDisOH), Sunyani, BA, Ghana, [5]University of Energy and Natural Resources, Sunyani, Ghana

Achieving the 2030 elimination target for onchocerciasis will require therapeutic strategies beyond ivermectin, and drug repurposing presents a rapid and cost-effective alternative. Ribonucleotide reductase (RNR), a key enzyme in *de novo* nucleotide biosynthesis, is critical for DNA synthesis and parasite survival and has been computationally predicted as a target for antimetabolite drugs like fludarabine phosphate and gemcitabine. This study molecularly confirmed and computationally characterized the small subunit of RNR in *Onchocerca volvulus* to validate its suitability as a repurposing target. Genomic DNA from adult worms was extracted, PCR-amplified, and sequenced to confirm the target gene (OVOC2713). The amino acid sequence was derived using ExPASy, and 3-D structural models generated using Phyre2. Functional domains and conserved motifs were identified with InterProScan and visualized in PyMOL. Sequence conservation across filarial species was assessed using Clustal Omega; structural integrity evaluated using ERRAT and Ramachandran plot analysis. Sequence analysis confirmed the identity of the RNR small subunit with 99% similarity to the predicted reference sequence. Structural annotation revealed conserved functional features: ferritin-like fold (res 59-85), canonical small subunit domain (res 1-102), and subunit A motif (res 36-85). Model validation demonstrated acceptable structural quality, with an ERRAT score of 88.85% and 86.2% of residues located within the Ramachandran favored regions. Comparative analysis showed high sequence conservation with other filarial parasites, including *O. flexuosa* (96.99%), *Brugia timori* (95.08%), and *B. pahangi* (90.96%). The study provides the first experimental confirmation and *in silico* structural validation of the RNR small subunit in *O. volvulus* parasites isolated from Ghana. The identification of conserved functional motifs essential for enzymatic activity supports RNR as a promising target for antimetabolite drug repurposing, offering a potential pathway to accelerate therapeutic development toward the global elimination of onchocerciasis.

6512

TOWARDS AN INTEGRATED POINT OF CARE (POC) DIAGNOSTIC KIT FOR CUTANEOUS LEISHMANIASIS: EVALUATION OF A RAPID DNA EXTRACTION METHOD

Yusr Saadi[1], Yasmine Zekri[2], Chaima Werchfeni[2], Rafeh Oualha[2], Hamed Chouaieb[3], Imen Khammeri[3], Akila Fathallah-Mili[3], Insaf Bel Haj Ali[1], Ikram Guizani[1]

[1]Laboratory of Molecular Epidemiology and Experimental Pathology (LR16IPT04), Clinical Investigation Center, Institut Pasteur de Tunis, Université de Tunis El Manar, Tunis, Tunisia, [2]Laboratory of Molecular Epidemiology and Experimental Pathology (LR16IPT04), Institut Pasteur de Tunis, Université de Tunis El Manar, Tunis, Tunisia, [3]Parasitology Department, Faculty of Medicine, Farhat Hached University Hospital, Université de Sousse, Sousse, Tunisia

Cutaneous Leishmaniasis (CL) is a major NTD in Tunisia and worldwide, where multiple *Leishmania* (*L.*) species coexist in complex settings. Accurate detection and species identification are essential for patient management. Although molecular diagnostics provide high sensitivity and specificity, their implementation remains limited including by the lack of rapid, simple, and field-adapted DNA extraction methods. This study evaluates a rapid DNA extraction kit, selected for its simplicity, speed and minimal equipment requirement, in comparison with a classical kit using a column and a centrifuge, to establish a POC diagnostic workflow combining rapid DNA extraction kit, multiplex PCR, and lateral flow detection (MxPCR-LF). Giemsa-stained slides (N=42) were prepared by spiking human blood with serial dilutions of *L.* promastigotes (5000-1 parasite/slide, triplicate). DNAs were extracted using the rapid kit and the reference kit. DNA quality for amplification was assessed by human β-globin PCR and parasites were detected by Lei70 PCR targeting the SSU rRNA. The extracted DNA is being evaluated with an efficient MxPCR-LF assay for simultaneous detection and species identification. The complete workflow is assessed on 60 skin smears (30 CL+ and 30 CL–). The rapid DNA extraction kit can be completed within 30 min with minimal handling and basic equipment (heat block and magnetic support), in contrast to a 2 h, multi-step, centrifugation-dependent reference kit. Amplifications by β-globin PCR have confirmed consistent DNA recovery and satisfactory quality. Lei70 PCR is currently used to compare analytical sensitivity. Compatibility with the MxPCR-LF assay is also under evaluation on spiked and clinical samples, which will allow performance and concordance measures with direct examination. Preliminary findings support the rapid DNA extraction kit for use in a simplified POC workflow for CL diagnosis, with gains in DNA processing time (2 h to 30 min), handling steps and equipment requirements. Even if the kit's costs are slightly higher, overall expenses are lower. DNA quality remains suitable for MxPCR-LF assay.

6513

SPATIAL AND TEMPORAL VARIATION OF MALARIA INCIDENCE IN CHILDREN UNDER 10 YEARS IN A PYRETHROID-RESISTANT VECTOR AREA IN SOUTHERN BENIN

Edouard Semako Dangbenon

Vector Control Product Evaluation Centre, BOUAKE, Côte D'Ivoire

This prospective cohort study of 1,806 children under 10 in southern Benin analyzed spatial and temporal dynamics of malaria incidence over 20 months in an area with pyrethroid-resistant vectors. Incidence ranged from 1.41 to 13.91 cases per 100 child-months. Spatial scanning identified significant transmission hotspots, with relative risks from 1.59 to 16.24. Cross-correlation analysis revealed a negative association with temperature (–0.56) and a positive association with rainfall (0.58). Multivariate analysis confirmed significant associations between incidence and altitude (aIRR 1.00, $p < 0.001$), soil type (aIRR 0.54, $p < 0.001$), and temperature (IRR 0.69, $p < 0.001$). The study demonstrates that integrating remote sensing, GIS, and spatial scanning methods enables precise identification of high-risk areas and associated environmental factors, thereby enhancing the cost-effectiveness of vector control interventions.

6514

MALDEEPSEQ: A HIGH-RESOLUTION TARGETED ULTRA-DEEP SEQUENCING FRAMEWORK FOR MALARIA GENOMIC SURVEILLANCE

Yanka Salazar[1], Antonio Rezende[1], Maria Carolina Puca[1], Sonja Lagström[2], Danielle Fletcher[3], Julian Muwanguzi-Karugaba[4], Colin Sutherland[5], Khalid Beshir[4], Maria Eduarda Mascarenhas[1], Jaime Louzada[6], Joseli Oliveira-Ferreira[7], Abdoulaye Djimde[8], Dinora Lopes[9], Taís N.D. Sousa[10], Jose Pedro Gil[11]

[1]Oswaldo Cruz Foundation, Belo Horizonte, Brazil, [2]Agilent Technologies, Solna, Sweden, [3]Agilent Technologies, Cheadle, United Kingdom, [4]LSHTM, London, United Kingdom, [5]London School of Hygiene & Tropical Medicine, London, United Kingdom, [6]Federal University of Roraima, Boa Vista, Brazil, [7]Oswaldo Cruz Institute, Rio de Janeiro, Brazil, [8]MRTC-USTTB, Bamako, Mali, [9]Institute of Hygiene and Tropical Medicine, Lisbon, Portugal, [10]René Rachou Institute/ Oswaldo Cruz Foudation, Belo Horizonte, Brazil, [11]Karolinska Institutet, Stockholm, Sweden

The emergence of drug-resistant *Plasmodium falciparum* strains remains a critical threat to global malaria control, requiring highly sensitive genomic tools to identify minority parasite subpopulations before resistant lineages expand. To address this, we developed and validated MalDeepSeq, a targeted ultra-deep sequencing framework designed for the comprehensive capture of full-length coding regions across 48 antimalarial resistance genes. The panel utilizes a custom-designed library of oligonucleotide probes for hybridization-based enrichment. This probe-based architecture ensures robust and uniform coverage across extensive genomic targets, enabling the identification of both known and novel mutations associated with drug response. To overcome the challenges of low parasite density and human DNA contamination, we integrated selective whole-genome amplification (sWGA) prior to the hybridization-capture step. The panel achieved high sequencing depth and breadth, reaching a median depth of 860× even in dried blood spot (DBS) protocols developed for resource-limited settings. Using 3D7:Dd2 mock mixtures, we evaluated the performance of variant callers, with Mutect2 demonstrating superior quantitative accuracy in recovering low-frequency signals near 9% compared to standard germline callers. Validations in diverse clinical samples from Brazil, Mali, and São Tomé and Príncipe confirmed the panel's capacity to capture extensive within-host genetic diversity and rare resistance alleles undetectable by conventional genotyping. Our integrated bioinformatics pipeline, implemented in Nextflow, enables reproducible and scalable execution, offering a cost-efficient, high-resolution alternative to whole-genome sequencing. By providing comprehensive coding sequence resolution and critical early warnings for treatment policy adjustments, MalDeepSeq represents a powerful innovation for genomic surveillance in heterogeneous transmission environments.

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MALARIA MOLECULAR SURVEILLANCE OF RESISTANCE TO SULFADOXINE PYRIMETHAMINE IN THE SENTINEL SITES ACROSS BURKINA FASO: PRELIMINARY RESULTS FROM BURKINA FASO NANOPORE SEQUENCING WITH THE NOMADS-MVP PROTOCOL

Oumou Aicha Zeina Djamila Zoure[1], Issiaka Issiaka Soulama[2], Oumarou Ouédraogo[3], Julien Nassandba Yanogo[3], Salam Sawadogo[3], Raissa Pousga ILY[3], Fabiola Saoudia Diara[3], Salif Sombié[4], Séni Nikièma[4], Dramane Zongo[3], Arsène Zongo[5], Abdou Azaque Zouré[3], Mulenga Mwenda[6], Daniel Bridges[7], Jason Hendry[8], Adama Zida[9]

[1]Research Institute in Health Sciences, Ouagadougou, Burkina Faso, [2]Institut de Recherche en Sciences de la Santé (IRSS), Ouagadougou, Burkina Faso, [3]Institut de Recherche en Sciences de la Santé, Ouagadougou, Burkina Faso, [4]Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso, [5]Robert Koch Institute, Centre for International Health Protection, Berlin, Germany, [6]PATH,

Lusaka, Zambia, Lusaka, Zambia, [7]PATH, Lusaka. Zambia, Lusaka, Zambia, [8]Max Planck Institute for Infection Biology, Berlin, Germany, [9]Université Joseph KI ZERBO, Ouagadougou, Burkina Faso

Sulfadoxine-Pyrimethamine (SP) continues to be the main component of Intermittent Preventive Treatment during Pregnancy (IPTp) and Seasonal Malaria Chemoprevention (SMC) in Burkina Faso. The emergence and spread of mutations in the *Pfdhfr* and *Pfdhps* genes of *Plasmodium falciparum* represent a growing threat to the effectiveness of these interventions. The establishment of malaria molecular surveillance is essential to generate evidence for public health decision-making. Three hundred blood samples were collected on filter paper (DBS) in 2024 from July to October through five sentinel malaria surveillance sites representative of the Sahelian (Kaya), Sudano-Sahelian (Koudougou, Koubri) and Soudanian (Bobo-Dioulasso, Kampti) malaria epidemiological facies of Burkina Faso. Out of the 300 blood samples colected, 122 were successfully sequenced for the *Pfdhfr* gene and 172 for the *Pfdhps* geneThe evaluation of mutations in *Pfdhfr* (N51I, C59R, S108N) and *Pfdhps* (S436A, A437G, K540E, A581G, A613S) was carried out using the Oxford Nanopore Technology (ONT) sequencing platform according to the NOMADS Minimal Viable Panel protocol. The analysis of mutations in the *Pfdhfr* gene reveals a high prevalence of individual mutations : N51I (85%), C59R (94%) and S108N (95%). The IRN triple mutant (N51I+C59R+S108N), a major resistance haplotype, represents 84% (n=103) of the isolates analyzed. Regarding the *Pfdhps* gene, the A437G mutation predominantes (87%), associated with S436A (58%), defining the double mutant SA+AG as the predominant haplotype (38%). The *Pfdhps* K540E mutation, a key driver of full resistance to SP, remains at a very low frequency (1%, n=2), while *Pfdhps* A581G is detected at 1%. No significant inter-facies epidemiological variation is observed at this stage of the analyses. These preliminary results show a high frequency of the triple mutant *Pfdhfr*-IRN and the emergence of double mutants *Pfdhps* in Burkina Faso. The low prevalence of K540E provisionally preserves the usefulness of TPIg-SP. A continuous monitoring of resistance to SP remains necessary to guide national intervention policies. 1

6516

PRECLINICAL DEVELOPMENT OF A TOPICAL LIPOSOMAL MILTEFOSINE FORMULATION FOR THE TREATMENT OF CUTANEOUS LEISHMANIASIS

Francesca Papera[1], Diego N. Rios[1], Monica Sanchez[1], Jesica Piovano[1], Eliana Martinez[1], Ma. Laura Guzman[2], **Dolores C. Carrer**[1]

[1]INIMEC-CONICET-UNC, Cordoba, Argentina, [2]Dpt. of Pharmaceutical Sciences, UNITEFA – CONICET - Universidad Nacional de Córdoba, Cordoba, Argentina

Cutaneous leishmaniasis (CL) is the most prevalent form of leishmaniasis, with an estimated 600,000-1 million new cases reported annually. The disease can cause pain, disfiguring scarring, and psychological distress, as well as substantial costs related to the management of adverse effects. Current treatments are inadequate, toxic and painful. A topical treatment would offer several important advantages, including self-administration, minimal or no systemic side effects, improved patient adherence, and lower production costs compared with parenteral treatments. We previously demonstrated the excellent efficacy (100% cure, sterilization of the lesion) of a topical formulation containing miltefosine and fluid liposomes in a murine model of CL. Promising results were also obtained during compassionate use in a first-in-human application of the formulation in a rural worker with treatment-resistant CL in northern Argentina. Here, we report advances in the pharmaceutical development of this formulation, following methodologies recommended by regulatory agencies such as the U.S. Food and Drug Administration (FDA) and the Administración Nacional de Medicamentos, Alimentos y Tecnología Médica of Argentina (ANMAT). These studies include the characterization of microbiological stability, rheological properties and spreadability, particle size, and the homogeneity of API concentration in the gel during storage in a multidose container over time. We also evaluated drug and lipid oxidation at different temperatures and assessed the effects of autoclave sterilization. Evaluation of local safety (skin irritation) as a function of concentration (after acute and chronic application) and pharmacokinetics following topical versus oral administration in animals is also underway. The formulation does not induce skin irritation at any of the concentrations measured, neither after acute nor chronic application, and most of the preliminary physical a chemical stability results are good. Still, other results suggest the inclusion of additional excipients may be necessary to optimize the formulation.

6517

SURVEILLANCE OF ANTIMICROBIAL RESISTANCE IN ESKAPEE MULTIDRUG-RESISTANT BACTERIA ACROSS PENINSULAR MALAYSIA AND SABAH, BORNEO.

Yee Qing Lee[1], Thanaporn Thongthum[2], Zhi Xian Kong[1], Nora G. Clearly[2], Wong Jia Haw[1], Sasheela Sri LaSri Ponnampalavanar[1], Andrew G. Letizia[3], Michael E. von Friken[2], Benjamin D. Anderson[2], Irina V. Etobayeva[3], Cindy Shuan Ju Teh[1]

[1]Universiti Malaya, Kuala Lumpur, Malaysia, [2]University of Florida, Gainesville, FL, United States, [3]U.S. Naval Medical Research Unit, Singapore, Singapore

Antimicrobial resistance (AMR) is a global health and military force health protection (FHP) threat with trends indicating increasing resistance in Malaysia, a hub of economic commerce and development. To better define local and operational risks within Malaysia, a 12-month surveillance study characterized the landscape of multidrug-resistant bacteria across four key medical facilities in Peninsular Malaysia and Sabah, Borneo. Human samples, including soft tissue, blood, urine, and body fluids, underwent AMR rapid testing confirmed by targeted PCR and whole genome sequencing. 419 ESKAPEE isolates were identified: (ESKAPEE) *Enterococcus faecium* (n=32), *Staphylococcus aureus* (n=67), *Klebsiella pneumoniae* (n=85), *Acinetobacter baumannii* (n= 97), *Pseudomonas aeruginosa* (n=30), *Enterobacter* spp. (n=31), and *Escherichia coli* (n=77). Of those, 75 isolates underwent whole-genome sequencing (WGS). *A. baumannii* (n=46) were dominated by the high-risk ST2 lineage *(82.6%)* across all hospitals and showed consistent carbapenem resistance, harbouring either the bla_{OXA-23} or bla_{OXA-51}, followed by 3rd generation cephalosporin drug-resistant *P. aeruginosa (n= 11).* 75% of *K. pneumoniae* (n=12) isolates carried bla_{NDM} *gene,* resistance to quinolones including ciprofloxacin, and aminoglycosides like streptomycin. *E. coli* isolates exhibited significant genetic diversity, suggesting varied evolutionary and transmission dynamics. 85% of all ESKAPEE isolates were nosocomial in origin contributing to a 31% patient mortality rate. Understanding the prevalence of drug-resistant pathogens in the region offers clinical guidance for selecting effective antibiotic therapies to treat endemic bacterial infections. This knowledge can inform regional public health and FHP planning, medical evacuation capabilities, and humanitarian assistance/ disaster relief (HA/DR) operations planning procedures.

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MALE REPRODUCTIVE TRAITS DRIVE VECTOR FITNESS IN *ANOPHELES FUNESTUS*

Ayesha S. Aswat[1], Yael Dahan-Moss[1], Lizette L. Koekemoer[2]

[1]1. Wits Research Institute for Malaria, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa; 2. Centre for Emerging Zoonotic and Parasitic Diseases, National Institute for Communicable Diseases of the National Health, Johannesburg, South Africa, [2]1. Wits Research Institute for Malaria, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa; 2. Centre for Emerging Zoonotic and Parasitic Diseases, National Institute for Communicable Diseases of the National Health, Johannesburg, Gauteng, South Africa

Anopheles funestus is a major malaria vector in sub-Saharan Africa, yet the role of male-biased genes in reproduction and population maintenance remains poorly understood. Characterising these traits may reveal host factors influencing vector fitness and identify novel control targets. Transcript *EZ976679*, sharing 99% sequence identity with a juvenile hormone esterase-like transcript, is highly expressed in males than females.

Given the role of juvenile hormone esterases in insect development and reproduction, this study investigated the functional role of *EZ976679* in *An. funestus*. Expression profiling of *EZ976679* was conducted across developmental stages, sexes, and dissected tissues using quantitative PCR, revealing male- and tissue-specific patterns. RNA interference-mediated knockdown was performed by nano-injecting double-stranded RNA into adult male mosquitoes. Transcript abundance was measured at multiple time points post-injection, and reproductive assays assessed mating success, fecundity and fertility. Longevity assays evaluated the effect of *EZ976679* knockdown on adult male survival. The results obtained indicate that *EZ976679* expression was male-specific, age-dependent and highly enriched in testes and accessory glands of 3-5-day-old males. RNA interference reduced transcript levels by >80% for 24 hours to 5 days, with partial recovery by 10-12 days. Knockdown males exhibited markedly reduced mating success, decreased female fecundity and shorter adult lifespan, while fertility remained unchanged. These findings reveal that *EZ976679* directly influences male reproductive performance and downstream female reproductive output. Therefore, transcript *EZ976679* is a male-specific transcript essential for reproductive success in *An. funestus*. As a male-specific transcript affecting mating success, fecundity and longevity, it represents a promising target for host-directed vector control. Understanding male-biased gene function opens new avenues to disrupt mosquito reproduction and reduce malaria transmission.

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CROSS-REACTIVE SARS-COV-2 ANTIBODIES IN PRE-PANDEMIC GAMBIAN SERA EXHIBIT FC-MEDIATED EFFECTOR ACTIVITY

Dawda Jobe[1], Ya Jankey Jagne[1], Natalie Barratt[2], Aji Fatou Touray[1], Hailey Hornsby[2], Sheikh Jarju[1], Madikoi Danso[1], Rhys Wenlock[3], David Hodgson[4], Lindsey Benjamin[2], Alansana Darboe[1], Beate kampmann[4], Thushan De Silva[2]

[1]Medical Research Council The Gambia at London School of Hygiene and Tropical Medicine, Banjul, Gambia, [2]University of Sheffield, Sheffield, United Kingdom, [3]London School of Hygiene and Tropical Medicine, London, United Kingdom, [4]Charité Centre for Global Health; Charité Universitqtsmedizin, Berlin, Germany

Pre-existing cross-reactive antibodies to SARS-CoV-2 have been reported in several African populations prior to the COVID-19 pandemic. However, the extent of their functional activity remains poorly understood. As Fc-mediated antibody functions are increasingly recognised as important components of protective immunity, we investigated antibody-dependent cellular cytotoxicity (ADCC) activity and IgG subclass distribution in pre-pandemic and pandemic samples from household cohort studies in The Gambia. SARS-CoV-2 anti-spike (S) and nucleocapsid (N) specific binding antibody responses were quantified in pre-pandemic (n=825) and COVID-19 pandemic samples (n=914) collected longitudinally across three 6-monthly sampling timepoints (2021-2022). Neutralising activity was assessed using a pseudovirus neutralisation assay, while ADCC activity was measured using a functional reporter assay. Associations between IgG subclass composition and ADCC activity were evaluated using correlation analyses. 21% of pre-pandemic samples were reactive to SARS-CoV-2 N protein, compared to 4% reactivity to S protein. Pre-pandemic N-reactivity increased with age, with 28% of adults >50 years showing seropositivity. No neutralising activity was detected in S IgG cross-reactive pre-pandemic sera. However, N IgG cross-reactive pre-pandemic samples displayed measurable ADCC activity that were moderately higher than those observed in pandemic sera. The response in pre-pandemic samples is associated with relative predominance of IgG3, whereas pandemic N-specific antibodies were largely IgG1 driven. Cumulative exposure to viral infections with age in The Gambia may have contributed to the high prevalence of crossreactive antiN IgG antibodies detected before the COVID19 pandemic. Although the source of this crossreactivity is unknown, these antibodies showed functional antiN ADCC activity in older adults, suggesting that Fcmediated antibody functions may influence antiviral immunity in African populations.

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ANTIMICROBIAL RESISTANCE IN COMMUNITY AND HEALTHCARE FACILITY DEATHS AMONG CHILDREN UNDER FIVE IN THE CHILD HEALTH AND MORTALITY PREVENTION SURVEILLANCE (CHAMPS) NETWORK.

Jim Seth Katieno[1], Annie Emanuels[2], Zachary Madewell[2], Aggrey Kitiezo Igunza[3], Victor Akelo[4], Hellen Muttai[4], Cynthia Gayle Whitney[5], Christopher A. Rees[2]

[1]Kenya Medical Research Institute, Kisumu, Kenya, [2]Emory University, Atlanta, GA, United States, [3]KEMRI - CGHR, Kisumu, Kenya, [4]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [5]Task Force for Global Health, Atlanta, GA, United States

Bacterial antimicrobial resistance (AMR) is a growing global public health concern, yet its burden in the community remains poorly understood. We compared AMR among children aged 0-59 months who died in community versus healthcare facility settings and assessed antecedent healthcare encounters. We conducted a multisite cross-sectional analysis using data from the Child Health and Mortality Prevention Surveillance (CHAMPS) network across seven sites in low- and middle-income countries. We included deaths among children aged <5 years with bacterial pathogens identified in blood or cerebrospinal fluid and available antimicrobial susceptibility testing. AMR was defined based on resistance among WHO priority pathogens. Among 1,704 deaths derived from 17,551 notified stillbirths and child deaths, 16.8% occurred in community settings and 83.2% in healthcare facilities. Neonates accounted for 59.6% of deaths, and infants/children 40.4%. Among community deaths, AMR was identified in 39.2% of neonatal and 32.5% of infant/child cases, compared to 84.8% and 76.3%, respectively, among facility deaths (P<0.001 for both). Overall, 81.5% of neonates and 62.8% of infants/children had resistance to at least one WHO priority pathogen. Resistance to third-generation cephalosporins was common, particularly among *Klebsiella pneumoniae* isolates, and was significantly higher in facility than community deaths (neonates: 92.3% vs 36.0%; infants/children: 83.4% vs 31.9%; P<0.001). Carbapenem resistance in *Acinetobacter baumannii* was high (93.6% in neonates; 82.6% in infants/children in facilities). Most community deaths with AMR had no documented prior healthcare encounters, suggesting considerable community acquisition or unrecognized exposure. Bacterial AMR was detected in both community and facility deaths, but the burden was substantially higher in healthcare settings, underscoring the dual need for strengthened community surveillance and intensified infection prevention and antimicrobial stewardship within healthcare facilities, particularly in high-burden under-five mortality settings.

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POINT-OF-CARE DETECTION OF MACROLIDE SUSCEPTIBILITY IN ENDEMIC TREPONEMATOSES BY LOOP MEDIATED AMPLIFICATION & LATERAL FLOW ASSAY

Jiem R. Ronas[1], Khalil J. Deveaux[1], LInda H. Xu[1], Hayden Z. Smith[1], Lorenzo Giacani[1], Joshua A. Lieberman[2]

[1]University of Washington, Seattle, WA, United States, [2]University of Washington, School of Medicine, Seattle, WA, United States

Detection and treatment of endemic treponematoses are limited by lack of clinical culture systems to grow or determine antibiotic resistance of the etiologic agents of yaws and bejel *Treponema pallidum* subsp. *pertenue* (TPE) and subsp. *endemicum* (TEN). Current treatment relies on single injections of azithromycin or long-acting penicillin. Resistance to macrolides develops quickly due to 23S rRNA point mutations A2058G and A2059G but is not widespread in TPE, unlike *T. pallidum* subsp. *pallidum* (TPP) where high prevalence of these alleles precludes the use of azithromycin to treat venereal syphilis. Thus, detection of azithromycin-susceptible and resistant strains is of significant individual and public health importance as azithromycin is the drug of choice for TPE. We developed three loop-mediated amplification (LAMP) assays for the detection of the A2058/A2059 macrolide-susceptible allele, and A2058G or A2059G resistant alleles in *T. pallidum* with detection by lateral flow assay (LFA). We evaluated

multiplexed detection of resistance-determining alleles with a separate LAMP primer set previously validated for detection of *T. pallidum* in our CLIA laboratory. Assay performance was evaluated using cultured TPP strains Nichols (A2058G/A2059G), SS14 (A2058G) and UW330B (A2059G). For all three primer sets, the 95% limit of detection was ~10 treponemes/reaction, equivalent to 300 treponemes/mL in swab media. The assay for the macrolide-susceptible allele was successfully multiplexed with the antibiotic-agnostic *T. pallidum* detection assay and had no cross-reactivity with strains with genotype A2058G or A2059G. Testing was reproducible across 3 operators at 10 treponemes/reaction. LAMP-LFAs for A2058G and A2059G performed similarly but could not be successfully multiplexed. Our results demonstrate a rapid, low-cost molecular test to predict macrolide effectiveness across *T. pallidum* subspecies. The assay can be performed with shelf-stable, lyophilized reagents. This approach is suitable for point-of-care treatment decision-making and could support monitoring emergence of drug-resistant yaws or bejel cases.

6522

CLINICAL IMPACT OF ANTIMICROBIAL RESISTANCE IN BLOODSTREAM INFECTIONS: A 13-YEAR NATIONWIDE MULTICENTER STUDY FROM THE UNITED ARAB EMIRATES

Madikay Senghore[1], Jens Thomsen[2], Najiba Abdulrazzaq[3], Wei Chen[1], Eveline Kaambo[1], Stefan Weber[4], Fouzia Jabeen[4], Godfred Menezes[5], Carole Moubareck[6], Abiola Catherine Senok[1], Dean Everett[1]

[1]Khalifa University, Abu Dhabi, United Arab Emirates, [2]Medics Labor AG, Bern, Switzerland, [3]Emirates Health Services, Dubai, United Arab Emirates, [4]Purelab, Abu Dhabi, United Arab Emirates, [5]Trinity Medical Sciences University, Kingstown, Saint Vincent and the Grenadines, [6]Zayed university, Dubai, United Arab Emirates

BackgroundBloodstream infections (BSIs) remain a major cause of hospital admission and death worldwide. We describe the epidemiology, outcomes, and resistance patterns of BSIs across the United Arab Emirates over 13 years, filling a critical data gap in the Gulf region.MethodsWe analyzed 50,457 unique BSI isolates collected between 2010 and 2022 from hospitals, centers, and clinics across all seven Emirates. We assessed pathogen distribution, acquisition source, antimicrobial resistance, multidrug resistance (MDR), and clinical outcomes. Multivariable logistic regression examined associations between pathogens and severe outcomes. ResultsWe identified a dual burden of severe BSI concentrated at the extremes of age. Adults aged ≥55 years accounted for 39.7% of cases and had frequent hospital-onset (50.4%) and the highest mortality (31.7%). Among children under 1 year old, we observed the highest ICU admission rate (43.6%), compared to 25.3% overall, and a high proportion of hospital-onset infection (54.4%). Although overall BSI incidence remained stable, candidemia increased significantly over time, and *Candida* species showed the strongest association with ICU admission and death. Carbapenem resistance was high in *Acinetobacter baumannii* (44.3%) and *Pseudomonas aeruginosa* (27.8%), and present in 17.3% of *K. pneumoniae*. Third-generation cephalosporin resistance affected nearly half of *E. coli* (47.3%) and 40.5% of K. pneumoniae. MDR infection was associated with significantly longer hospital stay and was concentrated in hospital-onset cases and critical care settings. In conclusion, BSIs in the UAE show a stable overall incidence but rising resistance and increasing candidemia. Severe outcomes cluster in neonates and older adults and are driven by hospital-associated and drug-resistant pathogens.

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MORTALITY AND LENGTH OF STAY ASSOCIATED WITH COMMUNITY-ACQUIRED *ESCHERICHIA COLI* AND *STAPHYLOCOCCUS AUREUS* BLOODSTREAM INFECTIONS: AN OBSERVATIONAL STUDY IN NINE LOW- AND MIDDLE-INCOME COUNTRIES

Sue J. Lee[1], On Behalf of the ACORN Investigators[2]

[1]MORU, Bangkok, Thailand

Antimicrobial resistance (AMR) disproportionally affects low- and middle-income countries. We calculated the burden of resistant and susceptible *Escherichia coli* and *Staphylococcus aureus* bloodstream infections (BSI) in ACORN2, a prospective clinical surveillance study in adults and children. Culture-confirmed community-acquired susceptible and resistant *E.coli* (third-generation cephalosporins: 3GC-S/3GC-R) and *S.aureus* (methicillin: MRSA/MSSA) patients with BSI were compared against blood culture negative patients (controls). Cause-specific hazard ratios (HR) were calculated for competing events of in-hospital mortality and discharge alive. Length of hospital stay (LOS) was calculated. From 22,802 blood cultures taken, there were 329 *E.coli* [177(53.8%) 3GC-R] and 363 *S.aureus* [132(36.4%) MRSA] BSIs. In-hospital mortality was 8.4% for controls, 20.1% for *E.coli* [20.3% 3GC-R; 19.7% 3GC-S] and 13.1% for *S.aureus* [12.2% MRSA; 13.7% MSSA]. 3GC-S but not 3GC-R *E.coli* BSI cases had higher adjusted hazard of death than controls [HR 1.73 (95% confidence interval 1.20-2.49) and 1.38 (0.99-1.94)]; risk of dying was the same for 3GC-S compared with 3GC-R infections (p=0.882). Neither MSSA nor MRSA BSI patients were more likely to die than controls [HR 1.33 (0.91, 1.93) and 1.00 (0.61-1.66)] nor MSSA versus MRSA (p=0.891). The hazard of discharge alive for both resistant and susceptible *S.aureus* and *E.coli* decreased significantly when compared with controls. LOS was 2 (0, 3) and 1 (-1, 3) day longer for resistant compared with susceptible infections, for *E.coli* and *S.aureus*. AMR infections were not associated with increased mortality in ACORN2. Drug resistant BSI had longer LOS compared with susceptible infections, but CIs encompassed 0 days. Prospective clinical AMR surveillance in Asia and Africa identified increased length of stay in patients with *E.coli and S.aureus* bloodstream infection, increased mortality in patients with *E.coli*, and no evidence of AMR-related mortality in either *E.coli* nor *S.aureus*.

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QUANTIFYING THE IMPACT OF ANTIMICROBIAL STEWARDSHIP PROGRAMS ACROSS MULTIPLE HEALTH CENTERS IN NEPAL AND LAOS

Jungseok Lee[1], Kshitij Karki[2], Phonevilai Santisouk[1], Yunjin Yum Yum[3], Wongyeong Choi[3], Rakchya Amatya[2], Bisekha Jaiswal[2], Geunhyeog Jang[1], Loungnilanh Manivanh[4], Deepak Bajracharya[2], Karishma Vaidya[5], Sanjit Shrestha[6], Vangnakhone Dittaphong[4], Nastu Sharma[7]

[1]International Vaccine Institute, Seoul, Republic of Korea, [2]G.T.A. Foundation, Kathmandu, Nepal, [3]Gwanak-gu, Republic of Korea, [4]Setthathirath Hospital, Vientiane, Lao People's Democratic Republic, [5]Paropakar Maternity and Women's Hospital, Kathmandu, Nepal, [6]B & B Hospital, Kathmandu, Nepal, [7]Manmohan Memorial Medical College and Teaching Hospital, Kathmandu, Nepal

Antimicrobial resistance (AMR), often referred to as the "silent pandemic," represents a notable threat to public health worldwide. Although antimicrobial stewardship programs (ASPs) are widely acknowledged as a primary intervention in addressing AMR, their impact remains inadequately studied—particularly in low- and middle-income countries (LMICs). The absence of robust evidence frequently leads to ASPs being deprioritized in public health agendas and excluded from the policymaking process, despite their cost-effectiveness. The current study aims to evaluate the statistically significant effects of ASP interventions at four tertiary-level health facilities in Nepal and Laos. A quasi-experimental design was utilized, incorporating a three-phase approach: baseline, intervention, and follow-up. The ASP intervention was introduced midway through the study

period, enabling comparison of key indicators before and after intervention. Interrupted time-series analysis was performed to assess the impact of ASPs on defined daily doses (DDD) per 100 bed-days and days of antibiotic therapy (DOT) per 1,000 patient-days. Patient-level medical records were collected from three health facilities in Nepal (B&B, Manmohan, Paropakar) and one hospital in Laos (STH), totaling 2,233, 1,939, 2,686, and 659 records, respectively. After the intervention, a statistically significant reduction (sudden shift) in DDD per 100 patient-days was observed in all Nepalese facilities except STH in Laos. Additionally, a sustained downward trend in DDD was noted at B&B during the follow-up phase. With respect to DOT per 1,000 patient-days, significant reductions were identified post-intervention at B&B and Paropakar, but not in the other two institutions. With ASP implementation costs ranging from $865 to $1,258 across the facilities, the intervention demonstrated excellent cost-effectiveness. The findings of this study will inform decision-makers, supporting advocacy, prioritization, and the expansion of ASPs. These results offer valuable evidence to promote similar cost-efficient interventions in resource-constrained settings.

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MICROBIAL DIVERSITY AND RESISTANCE TO LAST-RESORT ANTIBIOTICS IN CAMEROON: A NATIONWIDE CROSS-SECTIONAL STUDY OF ROUTINE DIAGNOSTIC DATA (2025)

Patricia Mendjime Fanemann[1], Teyim Pride Mbuh[2], Linda Augustine Juli Esso Endalle Lovet[3], Alexis Cyrille Saamene Juatio[4], Carine Agnès Mokam Youmbi[4], Mohamed Youssouf Mfouapon[4], Albert Bertrand Tchualeu Kameni[4], Essebe Ruth-Aimée Djapa Née Ngotty[4], Mirabelle Bayong Kyeng[5], Akoa Eyong Manda Esther[4], Emah Nguidjol Marguerite Inès[4], Hoche Black-Boy Emmual[4], Zra Armand[4], Shu Claudia Sirri[4], Ndong Alo'o Brice Marcel[4], Fadi Adokar Aicha[4], Mbang Soko Mathurin Florentin[4], Douka Sébastien Ledoux[4], Engamba Gaspar Cerges[4], Keka Fredrick Chi[4]

[1]Cameroon FETP, Yaounde, CE, Cameroon, [2]Teyim Pride, Douala, Cameroon, [3]MOH, Yaoundé, CE, Cameroon, [4]Cameroon FETP, Yaounde, Cameroon, [5]cafelt, Yaounde, Cameroon

The true burden of AMR in Cameroon remains insufficiently characterized due to fragmented laboratory infrastructure, necessitating reliance on empirical prescribing. This nationwide cross-sectional study established a clinical isolate profile to guide evidence-based stewardship. In June 2025, raw diagnostic data were extracted from public and private laboratory registers across Cameroon and consolidated into a line-list. Pathogen identification and susceptibility testing were performed by source laboratories using various protocols. We retrospectively re-interpreted results using EUCAST 2024 standards. MDR and XDR were defined by non-susceptibility to 1 agent in 3 and all but 2 antimicrobial categories, respectively. Carbapenem resistanc was defined by non-susceptibility to Meropenem, Imipenem, or Ertapenem. Data was analyzed using IBM SPSS statistic 25. Of 4,322 clinical samples, 890(20.6%) were positive for growth. The diversity profile comprised Gram-negative(39.4%), Gram-positive(34.6%), and atypical/fungal isolates(24.5%), with *E. coli*(22.4%) and *S. aureus*(11.0%) predominating. Regional analysis showed Gram-negative dominance in Littoral and Centre regions, while atypical/fungal isolates clustered in Western and Northern regions. Resistance profiling revealed 276(31.0%)MDR and 145(16.3%)XDR isolates. Notably, resistance to last-resort antibiotics was significant; 57(24.6%)isolates showed non-susceptibility to Meropenem, primarily *K. pneumoniae* and *E. cloacae*. High resistance rates were recorded for 3rd-generation cephalosporins(53.0%) and Cotrimoxazole(65.3%). The Littoral [58(40.0%), 22(38.6%)] and Centre [34(23.4%), 16(28.1%)] regions were Urban hotspots for XDR and carbapenem resistance cases respectively, while the Far North 83(14.3%) and North 61(10.5%) regions exhibited the highest Cotrimoxazole resistance. High burdens of MDR, XDR, and Carbapenem resistance necessitate urgent intervention. We recommend integrating laboratories into the national DHIS2 system, harmonizing diagnostic standards, and strengthening stewardship to preserve life-saving treatments.

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COMPARISON OF ORGANISM IDENTIFICATION AND ANTIMICROBIAL RESISTANCE IN CLINICAL ISOLATES USING CONVENTIONAL MICROBIOLOGY VERSUS WHOLE-GENOME SEQUENCING IN JOS, NIGERIA

Elizabeth Fernander[1], Joseph Nakah[2], Charles Asufi[2], Chanita Phichaphop[1], Uzal Umar[3], Antun Sanda[2], Ochala Sunshine[2], Bello Ozovehe Banimoh[2], Oludare Adedayo Odumade[1], Stephen Oguche[4], Tina Marye Slusher[1], Mark Ojogba Okolo[2], Beth Kristine Thielen[1]

[1]University of Minnesota, Minneapolis, MN, United States, [2]University of Jos, Jos, Nigeria, [3]Africa Centre of Excellence in Phytomedicine Research and Development Jos, Jos, ED, Nigeria, [4]Jos University Teaching Hospital, Jos, Nigeria

Antimicrobial resistance (AMR) is a leading global public health threat, disproportionately impacting patient morbidity and mortality in low- and middle-income countries. Surveillance is hindered in these settings by limited laboratory resources and funding. Because resistance patterns are often region-specific, hospital-level data is critical for effective clinical decision-making. This study compared traditional microbiology methods with next-generation sequencing, using portable and cost effective nanopore technology to identify bacteria and AMR patterns in clinical isolates from a tertiary hospital in Jos, Nigeria. Archival clinical samples collected at Jos University Teaching Hospital (JUTH) were revived for analysis. Organism identification was performed via manual biochemical assays and antimicrobial susceptibility testing via disk diffusion. In parallel, DNA was extracted using an ethanol precipitation protocol, followed by library preparation and barcoding for ONT sequencing. Genome data was uploaded to the University of Minnesota high-performance computing cluster and assembled using Flye. Assembled genomic contigs were uploaded to KmerFinder2 and ResFinder to identify organisms and AMR genes, respectively. Of the 52 bacterial samples sequenced, 43 were successfully sequenced and identified. Only 55% (24/43) of these NGS results matched the species identified by traditional microbiology. Disc diffusion assays of Gram-negative isolates revealed five multidrug-resistant (MDR) organisms, including four *E. coli* strains, and seven extended-spectrum beta-lactamase (ESBL) strains. Ongoing work includes comparing phenotypic disc diffusion results with genotypic AMR predictions based on sequencing data. This study highlights the variability between traditional microbiology techniques and NGS data, likely due to resource constraints affecting conventional identification methods in LMICs. These findings offers insight into the local antimicrobial resistance landscape at JUTH and demonstrate the potential for portable NGS to refine antibiotic management and improve outcomes for patients.

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BACTERIOPHAGE-MEDIATED TARGETING OF EXTENDED-SPECTRUM β-LACTAMASE (ESBL) *KLEBSIELLA PNEUMONIAE*: A STRATEGY TO REDUCE ANTIMICROBIAL RESISTANCE (AMR) BURDEN IN ROHINGYA CAMPS, BANGLADESH

Md. Shafiqul Islam, Golam Niaj Murshidi, Rifaquat Ahmed, Faria Marha, Md. Foysal Abedin, Md. Sakib Hossain, Md. Hajbiur Rahman, Ashrin Haque, Mohammad Atique Ul Alam, Md. Rafiqul Islam, **Zahid Hayat Mahmud**

International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh

Antimicrobial resistance (AMR) is a pervasive threat to global health, driven in part by extended-spectrum β-lactamase (ESBL)-producing *Klebsiella pneumoniae* conferring resistance to β-lactam antibiotics. In resource-limited settings such as the Rohingya refugee camps in Cox's Bazar, the spread of resistant bacteria is amplified through inadequate waste management and high population density. Therefore, the study aims to develop a potential bacteriophage-based treatment of resistant strains by isolating ESBL-producing *K. pneumoniae* and their corresponding

lytic phages to investigate phage lytic capacity, host range, stability and in vitro antibacterial and antibiofilm efficacy. Fecal sludge (FS) samples were collected from five FSTPs located within the refugee camp and ESBL-producing *K. pneumoniae* was isolated by using MacConkey and CHROMagar™ ESBL agar plates, and confirmed with 16s rDNA PCR. Bacteriophages specific to these bacteria were isolated, enriched and purified to high titers. Phage host range, lytic efficiency, and infection dynamics were evaluated along with phage stability under different pH and temperature. Finally, using crystal violet-based biofilm disruption assay, the antibiofilm capability of the phages was analyzed. Fifteen *K. pneumoniae* from FS samples and fifteen clinical isolates from a previous study were used for analysis. These isolates harbored high ESBL prevalence (bla_{CTX-M} 80%, bla_{TEM} 70%, bla_{SHV} 60%). Out of the isolated lytic phages, two were selected as they showed broad host range and optimally replicated at a Multiplicity of Infection (MOI) 1. Their stability was also confirmed in a wide pH and temperature range. Peak lytic activity up to 80% was observed at 6-8 hours post-infection. Finally, the antibiofilm activity showed reduction of biomass by 45.1%-84.4% for phage 1 and 20.9-76.2% for phage 2, demonstrating potent bacteriolytic and biofilm-disrupting activity. These findings accentuate phage-based therapeutics as a practical and effective strategy to combat resistant *K. pneumoniae*, especially in resource-limited settings while showing the need for further development.

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HIGH PREVALENCE OF MULTIDRUG RESISTANT*CAMPYLOBACTER* SPP. AMONG CHILDREN UNDER FIVE WITH ACUTE DIARRHOEA IN OUAGADOUGOU, BURKINA FASO

Alimatou Héma[1], Issa Tondé[1], Jean Zongo[1], Marguerite Edith Malatala Nikiema[2], Evariste Bako[3], Ange Badjo[4], Absatou KY BA[1], Moussa Sangaré[5], Abdoul-Salam Ouédraogo[6], Mahamoudou Sanou[1]

[1]Université Joseph KI-ZERBO (UJKZ), Health Sciences Training and Research Unit, Ouagadougou, Burkina Faso, [2]Laboratoire de Virologie et Biotechnologies Végétales, Institut de l'Environnement et de Recherches Agricoles (INERA), Ouagadougou, Burkina Faso, [3]Department of Biochemistry and Microbiology, Centre Universitaire de Tenkodogo, Tenkodogo, Burkina Faso, [4]Centre Muraz, Bobo Dioulasso, Burkina Faso, [5]International Center of Excellence in Research (ICER-Mali), University of Sciences, Techniques and Technologies of Bamako, Bamako, Mali, [6]Université Nazi Boni, Bobo-Dioulasso, Burkina Faso

Diarrheal diseases drive childhood mortality in sub-Saharan Africa, yet the *Campylobacter* burden and antimicrobial resistance (AMR) in Burkina Faso are underestimated due to diagnostic gaps. We quantified prevalence, species diversity, and AMR profiles among children under five in Ouagadougou. A prospective study (May 2023-April 2024) at the Centre Hospitalier Universitaire Pédiatrique Charles de Gaulle analyzed stool from 383 children with acute diarrhea. Samples were inoculated on selective agar (42°C, microaerophilic) and identified via biochemical profiling. Susceptibility was determined by disk diffusion (CA-SFM/EUCAST). *Campylobacter* spp. prevalence was 8.6% (n=33). While thermophilic species predominated (*C. jejuni* 42.4%; *C. coli* 21.2%), we identified a high proportion of *C. fetus* subsp. *fetus* (21.2%), an atypical finding at 42°C. Infection was significantly associated with the 12-23 month age group ($P < 0.05$). Notably, 100% of isolates demonstrated multidrug resistance (MDR) to ≥3 classes. Resistance reached 100% for ampicillin, tetracycline, and erythromycin (WHO first-line therapy). High resistance was observed for amoxicillin-clavulanic acid (93.9%) and ciprofloxacin (75.8%); gentamicin resistance was 42.4%. Co-resistance across five drug classes was common. These findings reveal a substantial *Campylobacter* burden with extreme MDR. Total failure of erythromycin and ciprofloxacin renders empirical protocols obsolete, leaving few therapeutic options. Results underscore an urgent need for revised clinical guidelines and integrated One Health surveillance to monitor resistance spillover from agricultural reservoirs to children.

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GAPS IN PRIVATE SECTOR READINESS FOR MALARIA CASE MANAGEMENT: A CASCADE ANALYSIS FROM ACTWATCH LITE STUDIES IN BENIN, CAMEROON AND NIGERIA

Keith Esch[1], Katelyn Woolheater[1], Manfred Accrombessi[2], Erica Wang[1], Paul Bouanchaud[1]

[1]PSI, Washington, DC, United States, [2]PSI, Cotonou, Benin

The private sector is an important source of care seeking for fever in Benin, Cameroon and Nigeria. These private sector outlets often operate outside routine national supervision and surveillance systems. We developed an outlet-level cascade framework to assess private sector readiness for malaria diagnosis, treatment and reporting. This is a secondary analysis of ACTwatch Lite data collected in Benin, Cameroon (Centre and Littoral regions) and Nigeria (Abia, Kano and Lagos states) between 2023 and 2024. ACTwatch Lite, which is funded by the Gates Foundation and implemented by Population Services International, is a streamlined malaria market study methodology examining malaria diagnostics, treatment, and provider practices using representative cross-sectional sampling. The cascade assessed three criteria: (1) whether outlets stocked any malaria diagnostic test, (2) stocked any artemisinin-based combination therapy, and (3) reported any malaria case data. In Benin, 54.1% (95% CI 43.2 - 64.6) of clinics and hospitals met all cascade criteria. In Cameroon, cascade completion was higher in Centre region (50.1%; 95% CI 39.3 - 60.8) than Littoral region (34.3%; 95% CI 27.0 - 42.4). In Benin and both Cameroonian study sites, no pharmacies completed the cascade. In Nigeria, cascade completion was highest in Kano (11.7%; 95% CI 9.4 - 14.4), followed by Lagos (3.0%; 95% CI 2.1 - 4.4) and Abia (0.5%; 95% CI 0.2 - 1.1). These disparities were driven by the higher proportion of patent and proprietary medicine vendors (PPMVs) in Kano completing the cascade (11.7%; 95% CI 9.1 - 14.8) than PPMVs in Lagos (0.2%; 95% CI 0.1 - 1.0) and Abia (0%). Across settings, major drops in readiness were observed at diagnostic availability and routine reporting. Substantial gaps remain in private sector readiness to provide quality malaria case management and contribute to routine surveillance systems. Strengthening diagnostic access and reporting mechanisms will be critical for improving malaria case management and surveillance.

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PERCEIVED FACILITATORS, BARRIERS, AND WILLINGNESS TO REPORT MALARIA DATA AMONG COMMUNITY PHARMACIES IN A LOW-TRANSMISSION SETTING IN GHANA : A CONTINGENT VALUATION EXPERIMENT

Harriet Affran Bonful[1], Collete Santah[2], Micheal Opoku-Mireku[3], Hainau Iddrisu[1], Victoria Anima Fordjor[1], Morrison Asiamah[1], Paul Boateng[4], Keziah Laurencia Malm[4], Adolphina Addo-Lartey[1], Livesy Naafoe Abokyi[5], Irene Kretchy[6]

[1]University of Ghana School of Public Health, Accra, Ghana, [2]Department of Health Services, Policy, Planning, Management and Economics, School of Public Health, University of Development Studies, Tamale, Tamale, Ghana, [3]School of Public Health and Allied Sciences, Catholic University of Ghana, Fiapre, Ghana, [4]National Malaria Elimination Program, Accra, Ghana, [5]Department of Health Services, Policy, Planning, Management and Economics, School of Public Health, University of Development Studies, Tamale, Accra, Ghana, [6]University of Ghana School of Pharmacy, Accra, Ghana

Effective malaria surveillance is essential for achieving elimination, particularly in low-transmission settings where community pharmacies often serve as the first point of care. Despite their potential, the integration of private-sector pharmacy data into Ghana's surveillance systems remains underexplored, with limited attention to pharmacy workers' perceptions, attitudes and willingness to report. This mixed-methods study was conducted in a low-transmission district in Ghana. A cross-sectional survey employing contingent valuation assessed willingness to report among 135 pharmacy workers across 65 pharmacies. In-depth interviews were conducted with 11 community pharmacists and 6 stakeholders from

community pharmacists' leadership and the National Malaria Elimination Programme. Quantitative data were analysed descriptively, and qualitative data using thematic analysis. Willingness to support malaria surveillance was high (87.4%), with 69.6% willing to contribute time, 17.0% both time and resources, and 0.7% resources only. Perceived barriers included lack of data-handling skills (34.8%), absence of integrated tools (12.6%), time constraints (40.7%), financial limitations (34.1%), regulatory issues (15.6%), behavioural challenges (38.5%), and patient data security concerns (34.1%). In-depth interviews reiterated key barriers: the absence of a legal and regulatory framework, lack of integrated patient-level data systems, exclusion of reporting from routine practice, workload pressures, confidentiality concerns, and perceived profitability risks. Facilitators identified include user-friendly reporting tools, clear guidelines, training, and linking reporting to licensure renewal within an enforced regulatory framework. Despite high willingness, structural, regulatory, and resource-related barriers limit pharmacy participation in malaria surveillance. Strengthening training, regulation, and system integration is critical for effective private-sector engagement in malaria surveillance.

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USING ROUTINE SUPERVISION DATA TO ASSESS CHANGES IN MALARIA CLINICAL MANAGEMENT, DIAGNOSIS, AND REPORTING IN MOZAMBIQUE

Helio Mucavele[1], Jordan Smith[2], Abuchahama Saifodine[3], Bernadete Rafael[4], Salimone Nhancume[1], Flavio Jordao Wate[5], Josea Ratsirarson[2], Carlos Alberto Guerra[6], Ellen Knowles[2], Guillermo A. Garcia[2], Antonio Mussa[1], Mércia Dimene[1], Eliana Ferreira[1]

[1]MCD Global Health, Maputo, Mozambique, [2]MCD Global Health, Hallowell, ME, United States, [3]Office of Foreign Assistance, U.S. Department of State, Maputo, Mozambique, [4]National Malaria Control Program, Maputo, Mozambique, [5]Long-Term Exceptional Technical Assistance Project (LEAP Global), Chester, MA, United States, [6]MCD Global Health, Quito, Ecuador

Supportive supervision is widely used to strengthen malaria service delivery, yet evidence from routine implementation data remains limited. This study presents a preliminary analysis of supervision data from health facilities in Manica Province, Mozambique, to assess changes in adherence to malaria diagnostic and clinical management (CM) guidelines, and reporting accuracy across repeated visits during ongoing program implementation. Adherence to guidelines was measured through the critical protocol steps outlined in the national supervision tool, and data quality was assessed by comparing facility registers and monthly reports. Data from 374 supervision visits between February 2023 and January 2026 were analyzed; facilities with only one visit or with missing data were excluded. Data included 89 facilities (244 observations) for CM, 77 facilities (223 observations) for rapid diagnostic test (RDT) guidelines, and 133 facilities (282 observations) for data quality assessments. At the first visit, adherence to RDT and CM guidelines was 51% and 54%, respectively. RDT adherence reached 66% by the third visit, 76% by the fourth, and 86% at ≥5 visits. In contrast, CM adherence declined to 35% at the second visit, then increased to 40% at the third, 67% at the fourth, and 70% among facilities with 5 or more visits. Facilities receiving four or more visits showed the largest improvements between first and last visit (45% and 47% in RDT and CM adherence), and 44% of facilities demonstrated improvement in both domains, although gains were not consistent across all facilities. Data quality also improved across successive visits, with the proportion of facilities achieving 80% or higher reporting accuracy increasing from 73% at early visits to 96% after four or more visits. Improvements occurred across successive supportive supervision visits during program implementation, with earlier gains observed in standardized diagnostic procedures than the more complex CM. These data can provide insights to guide adaptive program management, although variability across facilities underscores the need for targeted supervision strategies.

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DIGITALIZING SEASONAL MALARIA CHEMOPREVENTION TO ENHANCE DATA QUALITY AND USE, COVERAGE, AND PROGRAM EFFICIENCY: A CASE STUDY OF TURKANA CENTRAL, KENYA

Elizabeth N. Ayoma[1], Victor O. Osiare[1], Vyvyenne Chigboh[2], Suzanne Van Hulle[3], David Ekai[4], Wendy P. O'Meara[5], Emmah C. Kimachas[6], Beatrice Chepkemoi Rotich[7], Diana Menya[8], Joseph Kipkoech[9], Andrew Wamari[10], Branice Kisali[10], Keitany Kibor[10], Edwin Mbugua[1]

[1]Catholic Relief Services, Nairobi, Kenya, [2]Catholic Relief Services, Lagos, Nigeria, [3]Catholic Relief Services, Baltimore, MD, United States, [4]Turkana County Government, Turkana, Kenya, [5]Duke University, Durham, NC, United States, [6]Duke, Eldoret, Kenya, [7]Eldoret, Kenya, [8]Moi University, Eldoret, Kenya, [9]Moi University, [10]National Malaria Control Program, Nairobi, Kenya

Seasonal Malaria Chemoprevention (SMC) is a proven, cost-effective intervention for reducing severe malaria and mortality among children in high-transmission settings. In Turkana Central Sub-County, Kenya, SMC implementation in 2024 relied on manual, paper-based tools, limiting real-time visibility of coverage, data quality, and timely programmatic decision-making. These challenges affected the ability to promptly identify gaps and optimize service delivery. In 2025, the program transitioned to a digital data collection system, Kenya's electronic Community Health Information System (eCHIS), to strengthen data use, improve coverage monitoring, and enhance operational efficiency in a fragile, nomadic context. A digital SMC module was developed and fully integrated into eCHIS, enabling mobile-based data collection and reporting. Community Health Promoters (CHPs) were trained to register households, capture eligible children, and accurately record SMC administration. Built-in validation checks, automated aggregation, and real-time dashboards supported continuous monitoring, supervision, and rapid feedback at all levels of implementation. A comparative analysis assessed differences in coverage trends and data performance between the manual (2024) and digital (2025) rounds, alongside user experience through interviews with CHPs and Community Health Assistants (CHAs). Under the manual system, coverage fluctuated from 88% to 104%, with reporting delays and data gaps. Following digitalization, coverage improved steadily from 68% in Cycle 1 to 106% by Cycles 4 and 5, reflecting better tracking and follow-up. Digitalization reduced reporting lag, improved completeness, consistency, and accuracy, and was widely accepted, with 80% of CHPs finding it easy to use and 69% of CHAs reporting improved data quality and usability. Overall, eCHIS strengthened data use, coverage performance, accountability, and program efficiency, demonstrating the value of digital health systems in supporting data-driven malaria prevention in fragile and resource-constrained settings.

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BRIDGING BORDERS, BRIDGING DATA: A CROSS-BORDER MALARIA CONNECTIVITY FRAMEWORK FOR ANGOLA AND NAMIBIA THROUGH CENTRALISED ANALYTICAL PARTNERSHIP

Ernest Moyo[1], Susan Fred Rumisha[2], Ousmane Diao[3], Christina Ibrahim Myalla[4], Herieth Alexander Mboya[5], Yasini M. Kokoda[1], Punam Amratia[6]

[1]Malaria Atlas Project, Dar es Salaam, United Republic of Tanzania, [2]Malaria Atlas Project, University of Oxford, UK, OXFORD, United Kingdom, [3]Dar es salaam, United Republic of Tanzania, [4]Dar Es Salaam, United Republic of Tanzania, [5]Gainesville, FL, United States, [6]Perth, WA, Australia

Cross-border malaria transmission poses a fundamental challenge to elimination in southern Africa, yet national surveillance systems operate independently with incompatible data structures, different reporting frequencies, and no shared analytical platform. Along the 1,376 km Angola-Namibia border, this fragmentation obscures connectivity between Angola's high-burden southern provinces and Namibia's border districts, where imported cases constitute up to 77% of confirmed malaria and have increased nearly seven-fold since 2021. We propose a centralised

cross-border analytical framework, coordinated through the Malaria Atlas Project (MAP), which supports both national malaria programmes. MAP's position as a trusted technical partner to both countries creates a unique opportunity to serve as a neutral hub for data harmonisation, joint epidemiological analysis, geospatial modelling and evidence-based dialogue between programmes that otherwise have limited formal mechanisms for shared surveillance. The framework will harmonise Angola's monthly DHIS2 facility data with Namibia's weekly surveillance records -- which uniquely disaggregate local from imported cases -- into a unified cross-border dataset spanning shared administrative boundaries. Aligned temporal aggregation, and integrated spatial layers will enable analyses currently impossible within either national system alone: gravity-based mobility modelling, temporal cross-correlation of transmission dynamics, and identification of priority importation corridors. Critically, this work is designed not as a standalone research output but as the foundation for sustained cross-border engagement, generating shared evidence that both programmes can use to coordinate vector control timing, synchronise campaigns, and target resources at the highest-connectivity corridors. We present the rationale, data architecture, and engagement strategy for this framework, arguing that technical partnerships positioned between national programmes can bridge the analytical gaps that perpetuate cross-border transmission in elimination settings.

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TRANSITIONING MALARIA SURVEILLANCE TO ELECTRONIC INTEGRATED DISEASE SURVEILLANCE AND RESPONSE (EIDSR): EARLY IMPLEMENTATION EXPERIENCE IN ZANZIBAR

Saidi Mgata[1], Joseph Joachim[2], Richard Sebastian Silumbe[3], Bakar Shariff Fakih[4], Katelyn Woolheater[5], Maggie Afenu[6], Christian Lengeler[7], Paul Bouanchaud[8], Christopher Lourenco[9]

[1]Population Services International – Tanzania, Dar es Salaam, United Republic of Tanzania, [2]Swiss Tropical Public Health Insitute, Basel, Switzerland, [3]Dar es Salaam, United Republic of Tanzania, [4]Swiss Tropical and Public Health Institute, Allschwil, Switzerland, [5]Austin, TX, United States, [6]PSI, Lawrence, MA, United States, [7]Swiss Tropical & Public Health Institute, Allschwil, Switzerland, [8]PSI, Washington, DC, United States, [9]PSI, Hopedale, MA, United States

Timely and complete malaria surveillance data are essential to detect, investigate, and rapidly respond to infections in elimination settings. In Zanzibar, the malaria surveillance system initially emphasized health facility-based notification with subsequent follow ups. As malaria transmission has declined, surveillance needs have expanded to include comprehensive case notification, investigation and response activities across both active and proactive efforts. To address these needs, Zanzibar is transitioning malaria reporting into an electronic integrated disease surveillance and response (eIDSR) system as part of broader surveillance strengthening efforts. The eIDSR upgrade was designed to support endtoend malaria surveillance workflows, including facility-based case notification, household-level case and site investigation, reactive case detection, and targeted response activities both passive and pro-active. On completion of system configuration and User acceptance test, the system was deployed and piloted in 15 health facilities and 15 DMSOs in Unguja. The results indicate improvements in case follow ups and understanding of transmission sites and patterns. The transition also highlighted implementation challenges including infrastructure limitations, training needs, and integration with existing malaria surveillance workflows. Integration of malaria surveillance into electronic IDSR platforms has the potential to strengthen real-time data use for elimination programs. Lessons from the Zanzibar transition highlight practical considerations for countries seeking to modernize malaria surveillance systems through digital reporting

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IDENTIFYING THE NEXT GENERATION MALARIA MOLECULAR SURVEILLANCE ASSAY IN PUBLIC HEALTH INSTITUTIONS: A TARGET PRODUCT PROFILE

Francis Farai Chikuse

Africa CDC, Addis Ababa, Ethiopia

Malaria Molecular Surveillance (MMS) strengthen regional collaboration, skills and expertise transfer, and data sharing among public health and research institutions. The first target product profile (TPP) for public health laboratories has been developed as a global malaria community effort, led by the Africa CDC Pathogen Genomics Initiative (PGI) with support from experts with extensive experience in malaria molecular surveillance, public health policy, therapeutic efficacy studies, and malaria case management. The TPP for anti-malarial drug resistance (AMDR) detection will guide the development of MMS as a routine in the National Public Health Laboratories and Institutes. Target product profile The TPP supports national genomics surveillance strategies under the Africa CDC, while also supporting the primary health care and universal health coverage targets. Malaria is primarily managed at primary health care facilities, and rapid detection and mitigation of anti-malarial drug resistance (AMDR) will improve patient outcomes and access to the correct malaria control interventions. This Through a coordinated review process involving multiple technical experts, users across the continent, other relevant international stakeholders including World Health Organization (WHO) and developers, the objective was to identify assays that best align with the established TPP criteria and are therefore recommended for broader adoption. PfPHAST and NOMADS assays were closer to TPP standards for strengthening MMS in public health institutions in Africa are being piloted in 10 countries by Africa CDC. In conclusion, identifying simple and scalable assays for MMS in public health laboratories is a key step to making MMS routine. As countries expand genomic and molecular surveillance to support improved decision-making in malaria control and elimination, the need for robust, reliable, and standardized assays becomes increasingly central.

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WEEKLY SMARTPHONE SURVEILLANCE CAPTURES ADDITIONAL REPORTED MALARIA EPISODES AND VECTOR SIGNALS IN A RURAL HIGH-TRANSMISSION COMMUNITY COHORT IN ZAMBIA

Westone Hamwata[1], Caitlin Bond[2], James L. Sichivula[1], Mena Kazerounian[3], Douglas E. Norris[4], Jean-Bertin Bukasa Kabuya[5], William J. Moss[6], Matthew M. Ippolito[7]

[1]National Health Research and Training Institute, Ndola, Zambia, [2]Baltimore, MD, United States, [3]Johns Hopkins, Baltimore, MD, United States, [4]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [5]Tropical Diseases Research Centre, Ndola, 8, Zambia, [6]Johns Hopkins Univ, Lutherville, MD, United States, [7]Johns Hopkins University School of Medicine, Baltimore, MD, United States

Periodic household-based malaria surveillance—while key for standardized, longitudinal measurement—may miss episodes between visits and the potential for complementary, community-reported vector abundance remains underexplored. We implemented weekly smartphone-based surveillance (SBS) nested within a monthly longitudinal cohort in Nchelenge District, Zambia, a rural area of perennial high-intensity transmission. 32 of 65 cohort households (mean 5.6 occupants) received smartphones preloaded with a survey application and were trained in basic smartphone use and survey operation. Incidence from weekly self-reports was compared with malaria estimates from monthly in-person household surveys over the same period (3,656 person-weeks). Mosquito trap counts were compared with participant-reported vector abundance. The median weekly response rate was 53.3% (IQR 24.5-74.5%). Surveys captured 381 reported malaria episodes, corresponding to 5.4 episodes/person-year, versus 0.26 episodes/person-year from monthly household surveys. In a conservative sensitivity analysis imputing all missing weekly surveys as malaria-free, SBS incidence remained 2.4 episodes/person-

year. 90.5% of self-reported episodes were reported to be confirmed by RDT or microscopy, 70.9% of care-seeking episodes were to a community health worker, and 18.4% to a health facility. Participant reports of mosquito abundance relative to the prior week and neighboring households correlated with concurrent mosquito trap counts (Kruskal-Wallis p=0.04 and p<0.001), whereas a subjective ordinal scale of overall abundance did not. Weekly SBS captured substantially more reported malaria episodes than monthly household surveys, even under conservative assumptions, and showed that some participant-reported vector signals aligned with trap counts, whereas an ordinal measure of overall vector abundance did not.

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PERCEPTIONS OF ELECTRONIC CLINICAL DECISION SUPPORT TOOLS FOR PEDIATRIC DIARRHEA CARE IN MALI

Sarah Dallas[1], Adama Mamby Keita[2], Mahamane Djiteye[2], Baba Seydou Kanté[2], Ousmane Dembélé[2], Aparna Divaker Mangadu[3], Léna Mazza[1], Samba O. Sow[4], Melissa HARPER Watt[1], Daniel T. Leung[5]

[1]University of Utah, Salt Lake City, UT, United States, [2]Center for Vaccine Development - Mali, Bamako, Mali, [3]Texas Tech University, El Paso, TX, United States, [4]Center for Vaccine Development Mali, Bamako, Mali, [5]Univ of Utah School of Medicine, Salt Lake City, UT, United States

Globally, diarrheal diseases consistently rank among the top five causes of death for children under five. Accepted best practice for managing diarrhea in the absence of blood or suspicion of cholera is rehydration; however, in resource poor areas, antibiotics are often prescribed due to financial pressures, caregiver expectations, and diagnostic uncertainty. Inappropriate antibiotic use often results in adverse side effects and contributes to the growing global health concern of antimicrobial resistance. Electronic clinical decision support tools (eCDSTs) have the potential to enhance antibiotic stewardship and overall quality of care by increasing adherence to evidence-based treatment guidelines and reducing unnecessary antibiotic use. This qualitative study explored the perspectives of healthcare providers and patient caregivers in Mali regarding the perceived utility, feasibility, and acceptability of an eCDST for clinicians to support pediatric diarrhea management. Data were collected through in-depth interviews with 18 providers and 36 caregivers of pediatric patients, followed by four focus group discussions with providers, and subsequently analyzed using a rapid thematic analysis approach. Data revealed strong interest in an eCDST, highlighting its potential to improve efficiency and quality of care. Participants emphasized that successful implementation would require adequate provider training and community outreach, particularly engagement with village chiefs and women's groups to build awareness and support. Caregivers prioritized improvement of their child's condition and generally deferred to providers in determining the most appropriate course of care, whether or not it involved an eCDST. Overall, these findings suggest that an eCDST is perceived as useful, acceptable, and feasible for supporting the treatment of pediatric diarrhea in the Malian healthcare setting.

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MAPPING THE EVOLUTION OF AI-GENERATED CONTENT IN THE MALARIA JOURNAL: A FOUR-YEAR BIBLIOMETRIC AND TEMPORAL TREND ANALYSES OF SCOPING REVIEWS

Julia García, Fhabián S. Carrión-Nessi, Victor N. Mwangi, **Carlos J. Chaccour**

Universidad de Navarra, Pamplona, Spain

Scoping reviews are a type of evidence synthesis designed to systematically map the breadth of literature, identify key concepts, and highlight knowledge gaps on a given topic. Although established guidelines (PRISMA-ScR) require strict rigor to ensure transparency and reproducibility, the exploratory nature of these reviews makes them vulnerable to automated generation via LLMs. In this study, we conducted bibliometric and temporal trend analyses on the usage of AI in scoping reviews published in the Malaria Journal from 2022 to 2025. We analyzed the main text of 178 articles using Pangram, an independently validated AI-generated content detector with sensitivity and specificity >99%. We classified the reviews based on their proportion of AI content detected: exclusively human (0%), minimal generation (<25% AI), moderate generation (25-50% AI), and substantial generation (>50% AI). The bibliometric analysis identified Africa (n = 82, 46%) as the most frequent continent of origin for first authors, followed by Asia (n = 36, 20%) and Europe (n = 34, 19%). Seventeen percent (n = 30) of the reviews had some detectable AI-generated content despite not disclosing its use in the methodology. AI-generated text accounted for 7% of the total word count across all reviews. The temporal trend analysis showed that while 100% of the reviews in 2022 (n = 39) were written by humans, four out of each 10 reviews in 2025 (n = 22/56) contained AI-generated writing patterns. This exponential growth in AI integration corresponds to the release cycles of major LLMs, such as ChatGPT, Gemini, and DeepSeek. The absence of AI disclosure in these reviews highlights a lack of transparency that contradicts current recommendations on scientific integrity regarding the use of assistive technologies. These findings show that the "silent" integration of AI-generated text into malaria research is rapidly increasing, which could undermine the trust and rigor of global health findings. It is urgent that journals and editors implement rigorous verifications and mandatory disclosure standards to preserve the validity of evidence synthesis in the effort to eliminate malaria.

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TRENDS IN AI-GENERATED CONTENT IN THE LANCET REGIONAL HEALTH JOURNALS, 2020-2025

Victor N. Mwangi, **Carlos J. Chaccour**

Universidad de Navarra, Pamplona, Spain

The rapid adoption of large language models (LLMs) and generative artificial intelligence (AI) is transforming academic writing and scientific communication, raising concerns about the extent of AI-generated content in scholarly outputs and its implications for learning, research integrity, and policy-making. We analyzed 993 commentaries published in The Lancet Regional Health Journals (Africa, Americas, Europe, South-East Asia, and Western Pacific) between their launching in August 2020 and December 2025 using the Pangram AI detection tool (sensitivity 0.98, specificity 1.0) to quantify human-written, AI-assisted, and AI-generated content. An internal validation was conducted using 50 pre-LLM commentaries (2020-2022), comparing original texts, AI-assisted revisions, and AI-generated outputs produced with ChatGPT. All commentaries prior to 2022 were correctly classified as fully human-written. From 2023 onward, commentaries containing AI-assisted or AI-generated text increased from 10 in 2023 to 48 in 2025, an almost 5-fold increment over two years. In 2025, AI-generated content ranged from 4.5% in TLRH Europe to 45% in TLRH Africa. Although the overall publication volume in these journals increased 9.73% in 2023-2024 and 12.81% in 2024-2025, excluding AI-influenced commentaries reduced growth to merely 1.12% and 0.56%, respectively. Our Internal validation showed that Pangram accurately identified AI-generated content while maintaining zero false positives for human-written and lightly AI-assisted texts. These findings demonstrate a rapid increase in AI-generated and AI-assisted content, with a substantial proportion of recent publication growth attributable to AI use. This raises important considerations for global health research systems, including scientific integrity, knowledge production, and the potential influence of AI-mediated content on policy development, warranting caution as AI tools become increasingly integrated into academic processes.

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SHIFTING CLIMATE DRIVERS OF DENGUE EPIDEMICS IN PUERTO RICO, 2000-2024: A MACHINE LEARNING ANALYSIS

Zachary J. Madewell[1], Ariana Detmar[1], Carol Y. Liu[1], Forrest K. Jones[1], Jomil Torres Aponte[2], Melissa Marzan-Rodriguez[3], Gabriela Paz-Bailey[1], Laura E. Adams[4], Maile B. Thayer[4]

[1]Division of Vector-Borne Diseases, Centers for Disease Control and Prevention, San Juan, Puerto Rico, USA., San Juan, PR, United States, [2]Division of Epidemiology & Research, Puerto Rico Department of Health, San Juan, Puerto Rico, San Juan, PR, United States, [3]Office of Island Affairs, National Center for State, Tribal, Local, and Territorial Public Health Infrastructure and Workforce, Centers for Disease Control and Prevention, Atlanta, Georgia, USA, San Juan, PR, United States, [4]Division of Vector-Borne Diseases, Centers for Disease Control and Prevention, San Juan, Puerto Rico, USA, San Juan, PR, United States

Puerto Rico experiences recurrent dengue epidemics, but climatic conditions associated with periods of elevated dengue activity are not well characterized. We evaluated climate and seasonal drivers of week-level high dengue activity in Puerto Rico using routine surveillance data and machine-learning methods. We analyzed 1,300 epidemiologic weeks of Passive Arboviral Disease Surveillance System data from 2000-2024. The outcome was weekly dengue activity above the epidemic alert threshold, defined as observed weekly case counts exceeding the threshold for that epidemiologic week. Predictors included same-week and lagged El Niño Southern Oscillation indicators—the Oceanic Niño Index (ONI) and El Niño Modoki Index (EMI)—Standardized Precipitation Evapotranspiration Index (SPEI) measures of drought and wetness, local meteorology (minimum temperature, precipitation, wind), and Fourier terms for seasonality. We fit an XGBoost classifier to estimate exceedance risk and used SHapley Additive exPlanations to identify influential predictors. Of 1,300 weeks, 231 (17.8%) exceeded the epidemic alert threshold. Elevated dengue activity was more common following higher ONI values 3-6 months earlier, higher EMI about 3 months earlier, warmer minimum temperatures, and wetter conditions, whereas weekly precipitation totals showed weaker and less consistent associations. In the fitted model, ENSO metrics and SPEI were the dominant contributors to exceedance risk, with smaller but detectable contributions from local temperature, precipitation, wind, and seasonal terms. Over time, ENSO and SPEI explained most broad increases in modeled risk during epidemic periods, while local meteorology explained additional week-to-week variation around these broader patterns. These findings suggest that large-scale climate variability and drought/wetness conditions are key contributors to elevated dengue activity in Puerto Rico. Interpretable machine learning applied to routine surveillance data may help public health programs anticipate elevated risk and better target vector control and dengue response activities.

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THE EFFECTIVENESS OF VIRTUAL REALITY ON LEARNING OUTCOMES AND KNOWLEDGE RETENTION IN MALARIA EDUCATION AMONG MEDICAL STUDENTS

Pabhawit Nisagornsen[1], Hirotake Mori[2], Rapeephan Rattanawongnara Maude[3]

[1]Ramathibodi hospital, Bangkok, Thailand, [2]Juntendo University, Bunkyo-Ku, Japan, [3]Bumrungrad International Hospital, Bangkok, Thailand

Malaria is a major public health concern in Thailand, yet interactive learning materials for this topic are limited. Although Virtual Reality (VR) is increasingly used in medical education, there are few VR tools for tropical infectious diseases, and data on their application in Thailand is lacking. Methods: We conducted a prospective, randomized, parallel-group trial recruiting 4th year to 6th year medical students at the Faculty of Medicine Ramathibodi Hospital. Participants were randomly divided into 2 groups: one using educational VR content and the other using lecture slides; both were prepared by the researchers. Students completed a pre-test, viewed the assigned media, and then took a post-test and an opinion questionnaire. A follow-up test for knowledge retention was scheduled 2 months later. We compared score changes using a multilevel mixed-effects linear model.Results: Twenty-seven participants were included. The estimated marginal mean scores for the VR group were 6.80 (pre-test), 9.00 (post-test), and 7.93 (retention). The lecture group scored 6.17 (pre-test), 9.58 (post-test), and 7.51 (retention). Both methods significantly improved immediate post-test scores, but the improvement was smaller in the VR group compared to the lecture slides group (mean change +2.20 vs. +3.42, p = 0.032). However, the VR group tended to perform better in the retention test, although the interaction between the teaching group and time point was not statistically significant (p = 0.065). For learner perception, 86.7% of the VR group reported high engagement (Likert score 4-5) compared to 50.0% in the lecture group. Open-ended feedback revealed 60% of VR participants found the module uniquely engaging, though 20% requested more interactivity, while 67% of the lecture group preferred the slides' familiarity but desired more multimedia. In conclusion. though VR did not show better performance than traditional slides for immediate learning, it may serve as a useful supplement to boost engagement with the potential for improving knowledge retention.

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DOXYCYCLINE AS A FAR-FORWARD ADJUNCT ANTIVENOM: A LOW-COST STRATEGY TO MITIGATE ENVENOMATION INJURY AT ROLE 1 AND 2

Meaghan Rose, Heather Zamora, Daniel Arens, Yoon Yeop Hwang

Naval Medical Research Unit San Antonio, San Antonio, TX, United States

Envenomation is a significant occupational hazard for military personnel, a risk projected to grow with global operational expansion. Supporting far-forward, multi-domain operations requires broad-spectrum, shelf-stable antivenom. This study supports repurposing Doxycycline (DOX), a cheap, abundant therapeutic, to neutralize venom and prevent multi-organ failure in field care settings. We report the half-maximal effective concentration (EC50) of DOX and proteomic profiles for 18 snake species native to key operational theaters, including North America, Africa, and Asia. DOX inhibited Phospholipase A2 (PLA_2) and Metalloproteinase (SVMP) activity was measured *in vitro* using enzymatic assays. Percent venom activity was determined via area under the curve for DOX in relation to venom alone and significance determined by one-way ANOVA with Dunnett's multiple comparisons and a nonlinear regression with dose-response-inhibition (EC50) at 95% CI and n = 3. Venom proteomes were profiled using Ultra-Performance Liquid Chromatography Quadrupole Time-of-Flight Mass Spectrometry data screened against genus level libraries in ProteinLynx Global Server. EC50 of DOX against SVMPs was ≤47 µg/mL across all species tested (500 µg/mL venom). For PLA2, the EC50 of DOX was <85 µg/mL for rattlesnake and viper venoms (50 µg/mL), <112 µg/mL for copperhead, cottonmouth, krait, and coral snake venoms (12.5 µg/mL), and <60 µg/mL for cobra and sea snake venoms (6.25 µg/mL). Proteomic profiles for each venom were correlated with EC50 values to anticipate DOX's broader effectiveness. Our findings support the repurposing of DOX as a cheap, shelf-stable, and effective countermeasure. It is an ideal initial intervention to minimize tissue damage and organ failure until a casualty can reach a higher echelon of care. These results are intended to guide future in vivo studies, ultimately leading to the adoption of DOX as a broad-spectrum antivenom that enhances pharmaceutical readiness in far-forward operational environments.

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DIGITIZING PAPER-BASED MORTALITY RECORDS TO ADDRESS GENDER AND SOCIAL INEQUITIES IN BOTSWANA: IMPROVING CAUSE-OF-DEATH SURVEILLANCE THROUGH CLOUD-BASED OPTICAL CHARACTER RECOGNITION (OCR)

Jaemin Jeon[1], Shathani Mugoma[2], Mooketsi Molefi[3], Gormao Lee[1], Peter S. Larson[1], Douglas Wiebe[1]

[1]University of Michigan - Ann Arbor, Ann Arbor, MI, United States, [2]Botswana Police Service, Gaborone, Botswana, [3]University of Botswana, Gaborone, Botswana

Countries across sub-Saharan Africa are navigating a profound epidemiologic transition, as shifting demographics and changing disease ecologies produce rising burdens of chronic, cardiovascular, and injury-related mortality alongside persistent infectious disease. Yet cause-of-death surveillance infrastructure in many settings remains designed primarily for infectious disease tracking, creating systematic blind spots for non-communicable and injury-related outcomes, many of which carry infectious antecedents. In Botswana, clinicians and forensic experts complete standardized paper forms that are rarely digitized, generating a bottleneck that prevents tracking of the evolving mortality landscape and obscures important gender and socioeconomic inequities in cause-of-death. To overcome this barrier, we developed a cloud-based optical character recognition pipeline to process forensic pathology reports from the Botswana Police Service. The workflow utilizes query-based extraction aligned directly with an existing data entry system, enabling automated, variable-level extraction into a secure database. In a pilot evaluation of 20 forensic records, the custom extraction adapter achieved an overall F1 score of 58.8%, with 61.3% precision and 56.5% recall. Compared to manual data entry requiring an average of 6.0 minutes and $2.00 per record, the automated workflow projects a 58% reduction in processing time and a 65% reduction in costs. While commercial cloud processing of sensitive mortality data raises concerns of patient privacy and data sovereignty, these can be mitigated through privacy-compliant APIs or localized offline OCR models. By shifting the burden from exhaustive manual entry to a streamlined verification workflow, this scalable system accelerates generation of high-quality, actionable data across all causes-of-death. This platform equips public health authorities to surveillance the epidemiological transition in real time, identify spatial and demographic mortality patterns, and design interventions that reflect the evolving complexity of population health in transitioning economies.

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NATIONAL MALARIA DATA REPOSITORY (NMDR): CENTRALIZING DATA TO ENABLE IN-DEPTH ANALYSIS AND TARGETED INTERVENTIONS IN MALI

Boubacar Sidiki Maiga[1], Cheikh Gassama[1], KEITA Fatoumata[1], Jean Baptise Kamate[1], Alassane BANGOURA[1], Suzanne Van Hulle[2], Yameni Chrestien[3], Mady Cissoko[4], Ibrahim Alkamiss CISSE[4], Aissata Kone[4]

[1]Catholic Relief Services - CRS, Bamako, Mali, [2]Catholic Relief Services, Baltimore, MD, United States, [3]Catholic Relief Services, Dakar, Senegal, [4]National Malaria Control Program - Mali, Bamako, Mali

Mali, a country with a high malaria burden, suffers from fragmented information systems that hinder data use for decision-making. To address this, the National Malaria Control Program, in collaboration with CRS, developed the National Malaria Data Repository (NMDR) to integrate and harmonize multiple data sources. A structured approach combined data mapping, modeling, and deployment of automated ETL pipelines for multi-source data integration and standardization. The NMDR integrates 17 modules fed by DHIS2 (routine and campaign data), climate data, national surveys (EIPM, EDS), and Excel databases from the past 10 years. It includes several programs (including 3 entomological and vector control programs), over 2,300 data elements, and 377 indicators via an interoperable Open Hexa architecture. Results show 99% data centralization and 100% coverage of priority indicators in a single platform. This integration improved cross-source consistency, reduced data access delays, and standardized national indicators, enabling advanced analyses such as spatio-temporal trends, correlations between climate variables and malaria incidence, and seasonality patterns. These analyses directly supported decision-making, including stratification, geographic targeting of interventions, and optimization of seasonal campaigns. The NMDR demonstrates that an interoperable data warehouse is an effective lever for strengthening data utilization and surveillance system performance in resource-limited settings.

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FINDING INSPIRATION IN GLOBAL HEALTH LEARNING: ENGAGEMENT AND IMPACT OF AN ONLINE, OPEN-ACCESS TROPICAL MEDICINE CURRICULUM

Jorge Cardenas-Alvarez[1], Rachel J. Gordon[1], Shauna Gunaratne[2], Daniel Griffin[3]

[1]Columbia University Irving Medical Center, New York, NY, United States, [2]Bronx, NY, United States, [3]Parasites Without Borders, Saint Augustine, FL, United States

Learning tropical medicine in non-endemic areas is challenging because the number of allocated hours for teaching is low, cases are infrequent and structured training is costly. To bridge this educational gap, The Tropical Medicine Curriculum (TMC) was created - an open access, multimedia, learning platform that provides an organized framework in high yield topics of tropical medicine. We assessed the level of engagement and impact of this platform as an educational tool. TMC was embedded on the Parasites Without Borders website. It was organized by taxonomical groups, each comprising of varying numbers of lessons and revised by ≥1 expert editor(s). Lessons were asynchronously delivered, each with a diverse mix of multimedia resources such as text, videos, pictures, infographics, podcasts and self-assessment e-modules. Quantitative metrics were obtained via Google Analytics 4. TMC was implemented on Sep28, 2025. By Mar25, 2026, the platform held 37 lessons across 7 groups. Five authors and 34 editors from 11 countries contributed to the platform. TMC received 18,170 views, of which 5,990 (32.9%) were active users (viewed≥2 pages or stayed on site>10 seconds) and 29,451 user interactions (clicks/video plays). Highest engagement (#views/%active users) was seen in the lessons: All courses (7,286/57.6%), Nematodes101 (1,422/63.4%), Intestinal Nematodes (1022/57.8%), Intestinal Protozoa 101 (513/69.0%), Cestodes101 (507/65.7%), Blood and tissue protozoa 101 (482/63.3%), Tinea versicolor (476/69.9%) and Malaria I (400/59.5%). TMC was meaningfully accessed (≥10 active users) across 73 countries. The highest engagement rates (35.8-61.1%) were seen in the USA, UK, Philippines, Colombia, Italy, Canada, Germany and India. Most users accessed TMC directly (51.1%) or via organic/social media referrals (38.0%). TMC is an innovative educational platform with high demand and meaningful engagement. Important challenges remain, as evaluation metrics may not accurately reflect learning outcomes. Future directions include evaluating its learning outcomes and integrating into existing under- or graduate medical education programs.

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INNOVATIVE DATA MANAGEMENT FOR SENSITIVE DATA COLLECTION IN RURAL GAMBIA: IMPLEMENTATION OF AUDIO COMPUTER-ASSISTED SELF-INTERVIEWING (ACASI IN THE PHASE SURVEY

Mamadou Sk Jallow[1], Bai Lamin Dondeh[2], Mehrab Karim[3], Adedapo SK Bashorun[4]

[1]Arden University, LONDON, United Kingdom, [2]Medical Research Council, Banjul, Gambia, [3]Medical Research Council, Banjul, Gambia, [4]Medical Research Council, Gambia

Collecting high-quality data on sensitive topics such as sexual behaviour in low-resource and low-literacy settings presents challenges related to accuracy, confidentiality, and completeness. The Prevalence of Human Papillomavirus, Antimicrobial Resistance, and Sexually Transmitted Infection

Estimation (PHASE) survey in The Gambia implemented innovative data management approaches to address these issues. The survey targets approximately 10,230 female participants aged 15-49 years. Data were collected using an Audio Computer-Assisted Self-Interviewing (ACASI) system on the SurveyCTO platform, enabling offline data capture in settings with limited internet connectivity. The system incorporated multilingual audio in five Gambian local languages, with visual aids to support low-literacy participants. Automated skip logic, real-time validation checks, and standardized questionnaires enhanced data quality. Data governance included expert review and Community Advisory Board validation.Results:ACASI improved data completeness and consistency by reducing interviewer bias and minimizing entry errors. Data were encrypted in transit and at rest, with unique identifiers ensuring confidentiality. Regular synchronization to a central server enabled real-time monitoring, automated query generation, and timely resolution of inconsistencies. Internal consistency checks, including repeated questions and randomized responses, further improved reliability. In conclusion, integrating culturally appropriate tools with robust data management systems can significantly enhance the quality, security, and reliability of sensitive data collection in low-resource settings. This approach provides a scalable model for similar epidemiological studies.

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ADVANCING HEALTHTECH FOR IMPACT: A SCOPING REVIEW OF ARTIFICIAL INTELLIGENCE IMPLEMENTATION FOR SKIN-RELATED NEGLECTED TROPICAL DISEASES (2021-2026)

Ngozi Murphy-Okpala[1], Chinwe Chika Eze[2], Ogochukwu Bielonwu[3], Ngozi Ekeke[4]

[1]Vertex Health Limited, Abuja, Nigeria, Abuja, Nigeria, [2]PRIME Data Nexus, Enugu, Nigeria, [3]ValueDrive Strategic Solutions Ltd., Abuja, Nigeria, [4]DAHW-German Leprosy and Tuberculosis Relief Association, Wurzburg, Germany

Artificial intelligence (AI) offers transformative ICT solutions for decentralizing healthcare in low-and-middle-income countries (LMICs). However, real-world implementation is yet to scale at health-system level. This review followed PRISMA-ScR guidelines to map the extent of AI applications for skin-related Neglected Tropical Diseases (Skin-NTDs) for insight on implementation approaches and gaps. We searched PubMed, Scopus, WHO Global Index Medicus and IEEE Xplore for LMIC-focused studies published between 2021 and 2026. Our search yielded 916 papers screened to 51 peer-reviewed articles that met the inclusion criteria. Data extraction and thematic analysis were structured using the Non-adoption, Abandonment, Scale-up, Spread, and Sustainability (NASSS) framework to evaluate their implementation. Most simultaneously addressed multiple skin-NTDs but Leprosy (n=19) and Leishmaniasis (n=10) had the largest distinct category, with zero standalone papers for conditions like Scabies, Lymphatic Filariasis or Yaws. Majorly proof-of-concept papers, solutions were heavily skewed toward decision support for diagnosis, with limited use case for rehabilitative care, and none for psychosocial support. Core technology spanned through classic machine learning to edge AI however, delivery format was overwhelmingly one-sided with >90% of tools relying on image-based computer vision via smartphones or microscopy. Remarkably, no study used audio or voice-enabled conversational AI. Applying NASSS framework highlights critical implementation gaps as neglecting integrated service delivery and care continuum neglects the global roadmap to eliminate NTDs. Also, primary reliance on text and visual interfaces ignores the target users' practical literacy hurdles, restricting equitable adoption. Future AI research must go beyond diagnostics to significantly influence global health. Developing accessible delivery channels like voice interfaces, expanding clinical scope to integrated care, and increasing the pool of longitudinal causational studies will produce reliable evidence required to impact policy and practice.

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FROM PLANNING TO IMPACT: THE CAMPAIGN INFORMATION MANAGEMENT SYSTEM AS A CONFIGURABLE PLATFORM FOR CLOSING THE LOOP ON PUBLIC HEALTH INTERVENTION DELIVERY

Duncan McFarland[1], David Galick[2], Jeremias Nzamio Mba Eyono[2], Carlos Alberto Guerra[3], Guillermo Alberto Garcia[1]

[1]MCD Global Health, Hallowell, ME, United States, [2]MCD Global Health, Malabo, Equatorial Guinea, [3]MCD Global Health, Quito, Ecuador

Public health programs generate substantial data across planning, field operations, surveillance, and impact evaluation, yet most lack the infrastructure to interconnect these data streams into a coherent system that informs decisions in real time. The Campaign Information Management System (CIMS) was developed by the Bioko Island Malaria Elimination Project (BIMEP) to close this loop across all stages of public health intervention delivery. In continuous operation since 2014, CIMS has evolved into a fully configurable platform applicable to any intervention requiring population-level planning, multi-level data collection, supervisory oversight, program analytics, and longitudinal impact measurement. The platform integrates four connected functions: mapping and sampling target populations at any geographic or administrative scale using open source data; configuring deployment plans with pre-built indicators, coverage targets, and real-time dashboards; guiding field workers offline with map interfaces automatically updated to show completed, pending, and available targets at household, facility, or individual level; and triggering automated WhatsApp messages to beneficiaries after service delivery and to field workers on performance and available work. A map interface allows field workers to navigate spatially to targets, monitor workload, and coordinate across teams. A consent-based facial recognition system is also used for individual tracking as required by certain campaigns. Nearly two million records have been accumulated across vector control, malaria surveys, household censuses, LLIN distribution, larval source management, supervisory quality control, cervical cancer screening, and vaccination campaigns in Equatorial Guinea and Madagascar. CIMS demonstrates that a single configurable platform grounded in real program data can serve as the operational and analytical backbone for any public health campaign.

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IMPLEMENTING DIGITAL DATA COLLECTION FOR ROUTINE IMMUNIZATION AT COMMUNITY LEVEL: LESSONS LEARNED IN MADAGASCAR

Liyu Teklemichael[1], Duncan McFarland[1], Jerry Rakotozafy[2], Rija Fanomeza[2], Ellen Knowles[1], Guillermo Alberto Garcia[1], Carlos Alberto Guerra[3]

[1]MCD Global Health, Hallowell, ME, United States, [2]MCD Global Health, Antananarivo, Madagascar, [3]MCD Global Health, Quito, Ecuador

Integrating digital data systems into existing community health programs in remote settings requires navigating multitier data chains, connectivity gaps, and distributed workforces that cannot be reached through conventional training approaches. Here we describe lessons learned from deploying the Campaign Information Management System (CIMS), a Spatial Decision Support System (SDSS) developed by MCD Global Health. The CIMS was developed to plan, monitor, and evaluate public health interventions. In Madagascar, it was deployed as a digital data collection and dashboard layer within a community-driven vaccination program already operating across 12 districts, introduced mid-program rather than as a planning tool. The program relies on approximately 700 community agents collecting data on paper, who feed data upward to approximately 70 technical assistants (TAs) distributed across the 12 districts, some remotely located, who in turn are supported by the MCD Global Health MEL team. Training TAs on digital data collection under conditions of power outages and limited internet and phone coverage required combining remote support with in-person field visits to the most remote locations. Each update to data collection forms triggered a new round of remote retraining across a geographically dispersed team with variable connectivity. Despite these constraints, CIMS

was successfully deployed across all 12 districts. Data from all community agents were captured through the TA network and dashboards became the primary monitoring and evaluation tool for program management. Deploying a configurable digital system into an existing program mid-stream is operationally feasible in remote settings but requires explicit planning for tiered training, iterative reconfiguration support, and physical presence where connectivity cannot be assumed.

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DEPLOYING A GAUSSIAN-PROCESS CONVOLUTIONAL NEURAL NETWORK HYBRID ARCHITECTURE THROUGH AN IOS-BASED UNCERTAINTY-AWARE GEO-INTELLIGENT MACHINE LEARNING PIPELINE FOR OPTIMIZING HOT SPOT PROBABILITY MAPPING FOR HABITATS OF *ANOPHELES* SP. USING "SEEK AND DESTROY": A LARVAL SOURCE MANAGEMENT PROGRAM IN MALANJE PROVINCE, ANGOLA

Payton M. Jachec, Sanjana Nallapati, Aarya P. Satardekar, Rishil Shah, Ismael Hoare, Benjamin G. Jacob

University of South Florida, Tampa, FL, United States

We developed a hybrid Gaussian-process convolutional neural network to identify *Anopheles* sp. habitats from spectrally interpolated Worldview-4 31-centimeter resolution signatures in Malanje Province, Angola. The framework combined deep convolutional feature extraction with probabilistic GP classification in a geo-intelligent pipeline for malaria surveillance and targeted vector control using S&D larval source management. Georeferenced *Anopheles* sp. habitat observations from field surveys were encoded as binary presence-absence labels. Multispectral Wv-4 imagery was preprocessed using atmospheric correction, cloud masking, radiometric normalization, and spatial co-registration. Environmental covariates including vegetation greenness index, surface temperature, elevation, and rainfall were stacked as input channels to enhance sensitivity to land-use-land-cover conditions associated with larval habitats. Fixed-size image patches centered on labeled locations were extracted and balanced to address class imbalance. A neural network learned latent spatial-feature representations from image patches. Data augmentation and dropout reduced overfitting through a stochastic spectral interpolator passed to a classifier capturing nonlinear relationships and quantifying uncertainty. A radial basis function kernel with sparse variational inference ensured scalability to the provincial level from a georeferenced Sentinel-2 site for mapping hot and cold spots. The model was trained end-to-end using variational inference optimized via adaptive moment estimation optimization and evaluated using eigen-Bayesian autocorrelation cross-validation to minimize spatial dependence bias. Metrics included area under the receiver operating characteristic curve precision, recall, and F1 score. The model revealed sensitivity and specificity approaching 100%. Posterior predictive means and variances generated *Anopheles* sp. habitat suitability maps and uncertainty surfaces enabling identification of high-risk areas for targeted field validation and adaptive vector control at the provincial level.

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QUALITY CONTROL AND ANALYSIS TOOLS FOR LARGE SCALE DATASETS GENERATED BY FLUORESCENT MICROSPHERE IMMUNOASSAYS

Neeraja Venkateswaran[1], **Nishanth Parameswaran**[1], Viswanathan V. Krishnan[1], Kumar V. Udhayakumar[2], Kodumudi Venkateswaran[3]

[1]Tetracore Inc, Rockville, MD, United States, [2]Task Force for Global Health, Atlanta, GA, United States, [3]Tetracore Inc., Rockville, MD, United States

Multiplex bead assays (MBA) can simultaneously measure targets to multiple markers of interest. The adoption of the Luminex MBA in large surveys presents unique challenges due to its multidimensional data. After an assay run, quality control (QC) checks must be performed, datasets may be merged, and qualitative and/or quantitative data may be interpreted. This process can be laborious without the efficient tools to limit user error. In 2024, the United States Centers for Disease Control and Prevention published an open-source R Shiny application, *shinyMBA*. *shinyMBA* enabled Luminex MBA users to expedite QC processes and merge datasets from raw data files generated by xPONENT or Bio-Plex Manager software. Users can evaluate bead counts, median fluorescence intensity (MFI), and assay controls. Tetracore *shinyFIA*, that incorporates these features and added three modules: Internal Controls, Cutoffs, and Standard Curves. The modified app can be used in portable, standalone mode as well as online. Tetracore FlexImmArray tests use internal control beads to evaluate addition of sample, fluorescent reporter, non-specific binding, and instrument function for each well. The Internal Controls module determines whether each internal control bead meets the rules and flags samples that do not. The Cutoffs module allows users to select markers with predetermined cutoff MFI values. The app calculates within-batch and multi-batch counts of samples that are above and below the cutoff for each marker. The Standard Curve module creates 4- or 5-parameter logistic curves for single- or multi-marker input, as defined by the user. Curves can be fit based on custom % recovery criteria, and replicates can be excluded before re-fitting. Users can generate a single standard curve per marker across a multi-batch study or a curve for each batch. Back-calculation of standards and interpolation of unknown samples are also provided. These features will enhance data analysis workflows for Luminex MBA users by providing enhanced QC options and qualitative/quantitative summaries of large-scale datasets.

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SAVING FOR HEALTH: A COMMUNITY-DRIVEN FINANCING MODEL TO REDUCE DELAYS IN CARE SEEKING AND IMPROVE MATERNAL HEALTH OUTCOMES IN WESTERN AND ALBERTINE UGANDA

Sandra Abbo

Belgian Agency for International Cooperation, Kampala, Uganda

Out-of-pocket health expenditure remains a major barrier to timely healthcare access in Uganda, leading to delayed care-seeking, preventable maternal and neonatal deaths, and household impoverishment. With only about 1% of the population covered by health insurance, most households rely on direct payments, increasing vulnerability to financial shocks. The Saving for Health (S4H) initiative, implemented since July 2023 across ten districts in the Rwenzori and Albertine regions, addresses these barriers among informal workers by integrating health savings into mature Village Savings and Loan Associations (VSLAs). While VSLAs are widely used for livelihood investments, health emergencies are often unplanned for, forcing households to sell assets or take high-interest loans. The intervention targets established VSLAs (≥2 years), where trained Village Health Teams and Saving for Health Ambassadors mobilize and train groups through a five-day curriculum on financial literacy, savings, and loan management, culminating in the S4H model. Members save a minimum of UGX 1,000 weekly and can access zero-interest loans of up to twice their savings for health emergencies, repayable within three months. The model prioritizes women's reproductive health while remaining inclusive of men. Early observations indicate reduced reliance on high-interest borrowing, improved preparedness for maternal emergencies, and decreased delays in seeking and reaching care. By embedding health financing within community systems, the S4H model demonstrates a scalable approach to strengthening financial resilience and improving access to care among vulnerable populations.

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PRELACTEAL AND NON-EXCLUSIVE BREASTFEEDING IN EARLY LIFE AND INFANT GROWTH FALTERING IN RURAL BANGLADESH

Farjana Jahan[1], Gabriella Rose Barratt Heitmann[2], **Abul kasham md. Shoab**[1], Anjuman Ara Rahman[1], Md. Mahmudul Hasan[1], Ayse Ercumen[3], Md Mahbubur Rahman[1], Jade Benjamin-Chung[4]

[1]International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Dhaka, Bangladesh, [2]Palo Alto, CA, United States, [3]North Carolina State Univ, Raleigh, NC, United States, [4]Stanford University, Stanford, CA, United States

Non-exclusive breastfeeding is common in low- and middle-income countries, yet drivers of mixed feeding and consequences for growth remain poorly understood. Using data from the CRADLE trial, we characterized early feeding practices and estimated associations with growth trajectories in 720 children with anthropometry assessed near birth and at 3, 6, and 12 months. We fit Poisson models to identify factors associated with feeding and linear mixed effects models to estimate associations between feeding and growth trajectories, adjusting for socioeconomic, maternal, prenatal, delivery, and neonatal factors, and child diarrhea. Within the first hour after birth, 61% of neonates breastfed and 76% received supplemental feeds including goat milk (26%), formula (26%), honey (11%), and cow milk (8%). From birth to 6 months, only 19% were exclusively breastfed, while 21% breastfed with water, 35% with formula, and 19% with animal milk. Home delivery (PR=1.25, CI 1.13, 1.39), delayed cord clamping (PR=1.21, CI 1.08, 1.35), traditional cord ointment (PR=1.11, CI 1.01, 1.21), and winter season (PR=1.25, CI 1.11, 1.40) were associated with higher prevalence of non-breastmilk feeds in the first hour. Non-breastmilk feeding in the first hour (PR=1.18, CI 1.06, 1.33) and fever during delivery (PR=1.08, CI 1.00, 1.17) were associated with higher non-breastmilk feeds to 6 months. Higher maternal age was associated with more animal milk and less formula in the first 6 months. Compared to exclusive breastfeeding, non-breastmilk feeding in the first hour was associated with steeper weight-for-age decline to 12 months (β = –0.021 Z/month; CI –0.042, –0.001), while animal milk (β = –0.025; CI –0.041, –0.009) and sugar water (β = –0.019; CI –0.035, –0.004) were associated with steeper length-for-age decline. Breastmilk + formula or animal milk to 6 months was associated with steeper weight-for-age increase (β=0.041 [0.004, 0.079]; β=0.044 [0.003, 0.085], respectively). Weight-for-length trajectories did not differ by feeding. In rural Bangladesh, prelacteal feeding and non-exclusive breastfeeding are common and are associated with growth faltering.

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USING CLIENT PERSPECTIVES TO STRENGTHEN MALARIA IN PREGNANCY SERVICES: FINDINGS FROM TARGETED SUPPORTIVE SUPERVISION IN GHANA

Abena A. Okoh[1], Sylvester Segbaya[1], Joseph Boye-Doe[1], Mark K. Ossom[1], Raphael W. Akpah[1], Abena N. Nartey[2], Hillarius Abiwu[2]

[1]PATH, Accra, Ghana, [2]National Malaria Elimination Programme, Accra, Ghana

Malaria in pregnancy remains a major cause of maternal anemia, low birth weight, stillbirth, and neonatal mortality in Ghana. In February 2026, targeted supportive supervision was conducted in 101 health facilities in the Ashanti and Savannah regions identified through routine DHIS2 data as having IPTp3 coverage below 50%, below the 2026 regional targets for Ashanti (62.0%) and Savannah (60.6%) and the national target (73.5%). Facility readiness was assessed using structured checklists, and exit interviews were conducted with 129 pregnant women attending antenatal care. Among respondents, 89.2% reported receiving some explanation of malaria prevention, but only 51.2% said the importance of IPTp-SP was clearly explained, while 41.1% said it was not clearly explained. Overall, 66.7% always felt comfortable asking questions, while 28.7% felt comfortable only sometimes. Fear of side effects was the main barrier to IPTp-SP uptake (60.5%), followed by insufficient explanation (15.5%); 5.4% believed it was unnecessary because they had no malaria symptoms. Women in Savannah were more likely than those in Ashanti to report not being told why IPTp-SP is important (20.0% vs. 4.8%; p=0.035) and less likely to feel comfortable asking questions (44.0% vs. 72.1%; p<0.001). Although SP availability (92.1%) and direct observed therapy implementation (93.1%) were high, only 36.6% of facilities had job aids or protocols, and 72.3% of providers were aware of WHO guidance on SP administration until delivery. In low-performing facilities, improving IPTp-SP uptake may depend less on commodity access and more on stronger client education. Interventions should emphasize benefits of IPTp-SP for mother and child, possible side effects, job aids, and context-specific communication to address concerns and improve adherence.

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IMPLEMENTATION FIDELITY OF AN INTEGRATED SERVICE DELIVERY INTERVENTION FOR RTS,S MALARIA VACCINATION UPTAKE IN SOUTHEASTERN LIBERIA: EVIDENCE FROM A POST-IMPLEMENTATION EVALUATION

Lisa Jesslyn Williams, Keith L. Gray

University of Liberia School of Public Health, Monrovia, Liberia

The RTS, S malaria vaccine was introduced in Liberia in April 2024, but sustaining uptake across the multi-dose schedule, particularly the 4th dose, remains challenging. An integrated service delivery intervention was implemented in 4 counties following a baseline assessment and human-centered design process. The intervention included triage of Road-to-Health booklets at facility entry, community health worker-led defaulter tracing, and co-delivery of Vitamin A, deworming, and insecticide-treated nets with the 4th dose. This study aimed to assess early implementation fidelity, service delivery outcomes, and caregiver experiences following implementation of the integrated intervention. A cross-sectional facility-based survey was conducted 2.5 months post-implementation using structured caregiver exit interviews (n=316) across 8 facilities in Maryland, Grand Gedeh, Grand Kru, and River Cess. Descriptive analyses estimated proportions of implementation fidelity, service delivery, and caregiver-reported outcomes, stratified by county. Triage fidelity was high (96%–100%), identifying 23%–39% of children as defaulters. Among those triaged, 31%–47% were referred for vaccination, and over 80% of referred children received vaccines the same day. Nearly all children attending facilities received a malaria vaccine dose, though uptake of complementary services was lower: vitamin A ranged from 12% to 49%, deworming from 10% to 23%, and insecticide-treated net distribution from 10% to 23%. Community health worker engagement was moderate, with 34%–78% of caregivers reporting reminders and 29%–51% reporting home visits. Caregiver satisfaction was high (>95%), and nearly all caregivers reported that triage at entry was helpful (>97%). The integrated intervention was implemented with high fidelity for triage and internal referrals, effectively identifying missed doses and facilitating same-day vaccination. However, lower uptake of complementary services and variability in community health worker engagement highlight key areas for strengthening to optimize impact and support scale-up.

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INTESTINAL HISTOPATHOLOGY ASSOCIATED WITH ENVIRONMENTAL ENTERIC DYSFUNCTION (EED) IN MALNOURISHED WOMEN OF REPRODUCTIVE AGE IN BANGLADESH

Md. Shabab Hossain

International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Dhaka, Bangladesh

There is limited knowledge on the histological characteristics of intestinal mucosa in women of reproductive age, the traditional gold standard for diagnosing environmental enteric dysfunction (EED). This study aimed to evaluate intestinal histological features in women of reproductive age with possible EED. A total of 129 malnourished women (body mass index [BMI] <18.5 kg/m²) and 42 healthy controls (BMI ≥18.5 kg/m²) enrolled in the Maternal EED study underwent upper gastrointestinal endoscopy.

Duodenal biopsy specimens were examined subject to histopathology to assess villous height, crypt depth, and inflammatory infiltrates in the lamina propria. Bivariate analysis was conducted to examine associations between malnutrition and histological findings. Chronic duodenitis was observed in 55% of malnourished women compared to 24% of controls (p<0.001). Villous atrophy was present in 22% of malnourished women versus 12% of controls, while crypt hyperplasia was identified in 19% and 7%, respectively. Based on histological assessment, 29% of malnourished women had mild EED, 10% moderate EED, and 16% severe EED. The prevalence of chronic duodenitis among women of reproductive age living in urban slums in Bangladesh is high and significantly greater among malnourished women compared to well-nourished controls. These findings highlight substantial intestinal mucosal abnormalities consistent with EED in this population and underscore the need for further investigation into its role in maternal undernutrition.

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WOMEN KNOWLEDGE EXPERIENCES AND ADAPTATION TO HIGH HEAT DURING PREGNANCY AND POSTPARTUM IN SIX LOW AND MIDDLE INCOME COUNTRIES

Shiyam Sunder[1], Hira Tariq[1], Sarah Saleem[1], Manujunath Somannavar[2], Avinash Kavi[2], Shivaprasad Goudar[2], Richard Derman[3], Rashidul Haque[4], William A. Petri[5], Archana Patel[6], Patricia Hibbered[7], Manolo Mazariegos[8], Nancy Kerbs[9], Antoinette Tshefu Kitoto[10], Melissa Bauserman[11], Elwyn Chomba[12], Waldemar Carlo[13], Monica Longo[14], Janet Moore[15], Elizabeth McClure[16], Robert Goldenberg[17], Blair Wylie[18]

[1]Aga Khan University, Karachi, Pakistan, [2]Jawaharlal Nehru Medical College, Belagavi, India, [3]Thomas Jefferson University, Philadelphia, PA, United States, [4]icddr,b, Dhaka, United States, [5]University of Virginia, Charlottesville, VA, United States, [6]Lata Medical Research Foundation, Nagpur, India, [7]Bostan University, Bostan, MA, United States, [8]Instituto de Nutrición de Centro América, Guatemala City, Guatemala, [9]University of Colorado, Aurora, CO, United States, [10]Kinshasa School of Public Health, Kinshasa, United States, [11]University of North Carolina School of Medicine, Chapel Hill, NC, United States, [12]Michigan State Univ, East Lansing, MI, United States, [13]University of Alabama at Birmingham, Birmingham, AL, United States, [14]Eunice Kennedy Shriver National Institute of Child Health and Human Development, Bethesda, MD, United States, [15]RTI International, Research Triangle Park, NC, United States, [16]UNC Chapel Hill, Chapel Hill, NC, United States, [17]Columbia University, New York, NY, United States, [18]Beth Israel Deaconess Medical Center, Boston, MA, United States

High heat is an emerging threat to maternal and newborn health in low-resource settings, but women lived experiences and adaptive responses remain poorly described. We analyzed two cross-sectional surveys nested within the Global Network Maternal and Newborn Health Registry across seven sites in Bangladesh, Democratic Republic of Congo, Guatemala, India, Pakistan, and Zambia from July 2023 to April 2025. Surveys were administered at pregnancy enrollment and 42 days postpartum. The antenatal survey included 23,483 women and the postpartum survey 19,680 women. During pregnancy, women commonly recognized heavy sweating (73.5%), tiredness/weakness (66.2%), and headache (55.5%) as heat-related symptoms, but fewer identified serious pregnancy risks including miscarriage (13.7%), stillbirth/fetal death (10.0%), poor fetal growth (11.2%), and preterm birth (11.9%). Commonly recognized adaptation strategies were seeking shade (83.2%), drinking more liquids (74.9%), and wearing lighter clothing (70.0%). Among postpartum respondents who experienced high heat, heavy sweating (74.7%), tiredness/weakness (65.7%), and headache (42.5%) remained common. Mothers also reported infant irritability (36.9%), disturbed sleep (35.9%), and rash (27.4%) during hot periods. Frequently reported infant protection strategies included keeping infants indoors (89.2%), avoiding outdoor exposure during the hottest times (82.8%), and keeping infants in shade (79.2%). Pregnant and postpartum women recognized common heat symptoms and used practical coping strategies, but awareness of serious maternal and newborn risks remained limited. Maternal and newborn programs should integrate climate-sensitive counseling, heat risk communication, and community-based adaptation support.

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REASSESSING THE BENEFITS OF NON-INDICATED ELECTIVE CESAREAN SECTION: EVIDENCE FROM A RURAL SUB-DISTRICT OF BANGLADESH

Rajib Biswas[1], Emily S. Gurley[2], Md. Sabbit Khan[1], Taukir Tanjim[1], Qazi Sadeq-ur Rahman[1], Mst. Lopa Moni[1], Mim Maria Rahman[1], Salma Afroz Shifa[1], Imran Hossain[1], Atique Iqbal Chowdhury[1], Shahana Parveen[1], Shams El Arifeen[1], Mohammad Zahid Hossain[1]

[1]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [2]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

Bangladesh has the highest cesarean section (CS) rate in South Asia (45%, 2022). Prior studies have reported no association or a protective association between elective CS and neonatal outcomes, potentially justifying this surgical surge. We investigated whether non-indicated, family-decided CS deliveries truly protect newborns compared to vaginal deliveries attended by skilled birth attendants (SBAs). We used prospective pregnancy surveillance data (2020-2024) from the Child Health and Mortality Prevention Surveillance (CHAMPS) health and demographic surveillance system in Baliakandi, a rural sub-district of Bangladesh. Outcomes of interest were neonatal morbidity (defined as requiring newborn hospitalization) and neonatal mortality. Among 21,314 live births, 64% (n=13,667) were CS deliveries. Of these, 7% (n=989) were family-decided elective CS. Among the 36% (n=7,647) vaginal deliveries, 43% (n=3,317) were attended by SBAs. We applied multivariable logistic regression adjusted for maternal sociodemographic and clinical characteristics. In initial adjusted models, family-decided CS was associated with significantly lower odds of neonatal morbidity (aOR 0.55, 95% CI 0.42-0.71) and mortality (aOR 0.42, 95% CI 0.20-0.78) compared to all vaginal deliveries attended by both skilled and unskilled birth attendants. However, when we compared to uncomplicated vaginal deliveries at facilities attended by SBAs, the difference in morbidity was no longer statistically significant (aOR 0.65, 95% CI 0.39-1.10). This suggests that the observed protective effect was attributable to skilled birth attendance rather than to CS itself. Furthermore, the apparent protective association was confined to private facilities (aOR 0.54, 95% CI 0.41-0.71), where 93% of family-decided CS occurred; no protective association was observed in public facilities (aOR 0.90, 95% CI 0.38-1.89). These findings highlight that expanding skilled birth attendant coverage for vaginal deliveries and enforcing evidence-based CS indications are essential policy priorities to reverse the trend of unnecessary cesarean sections in rural Bangladesh.

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DEVELOPING A DEMAND GENERATION STRATEGY FOR MATERNAL RSV AND GBS VACCINES IN KENYA: A SYSTEMATIC, BARRIER-DRIVEN PROCESS

Julie Nyanchama Nyamao[1], Lydiah Masakhwe Khalayi[2], Hellen Cherono Barsosio[3], Rose Jalang`o[4], Corrie Melvis[5], Barako Katelo[4], Benard Asuke Opiyo[2], Julius Ichodo Odero[6]

[1]Kenya Medical Research Institute (KEMRI), Nairobi, Kenya, [2]Kenya, [3]Kenya Medical Research Institute, Kisumu, Kenya, [4]National Vaccine Immunization Program, Nairobi, Kenya, [5]Kenya Medical Research Institute (KEMRI), Kisumu, Kenya, [6]Kenya Medical Research Institute, Busia, Kenya

Background: The anticipated introduction of new maternal vaccines against Respiratory Syncytial Virus (RSV) and Group B Streptococcus (GBS) in Kenya necessitates a comprehensive demand generation strategy to ensure equitable and sustained coverage. Methods: Through a structured ten-step participatory process, the Ministry of Health's National Vaccines and Immunization Program (NVIP) collaborated with the Kenya Medical Research Institute (KEMRI) and key stakeholders to develop a national demand generation strategy. The process encompassed multi-dimensional barrier analysis, target population identification, translation of barriers into strategic priority areas, intervention design, role assignment, indicator development, timeline sequencing, consideration of cross-cutting issues, and stakeholder validation. Results: Five strategic

priority areas were identified, each directly linked to a barrier cluster: (1) strengthening healthcare provider knowledge and capacity; (2) increasing community awareness and demand; (3) addressing vaccine hesitancy and misinformation; (4) improving access and convenience of maternal immunization services; and (5) strengthening health system support. For each priority area, specific interventions, responsible actors, measurable indicators, and phased timelines were established. In conclusion, this systematic, evidence-based and participatory process generated a comprehensive demand generation strategy for maternal RSV and GBS vaccines in Kenya. The methodology offers a replicable framework for other low- and middle-income countries planning introductions of novel maternal vaccines.

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POST DIARRHEAL NUTRITIONAL TRAJECTORIES AMONG MALNOURISHED CHILDREN AGED 6-35 MONTHS: A CLUSTERING AND MULTINOMIAL MODELLING APPROACH

Billy Ogwel[1], Alex O. Awuor[1], Brian O. Otieno[1], Raphael Ochieng[1], Richard Omore[1], Elizabeth Rogawski McQuade[2]

[1]KEMRI, Kisumu, Kenya, [2]Emory University, Atlanta, GA, United States

Although the co-occurrence of diarrhea and malnutrition is well documented, research has largely focused on the acute management of diarrheal illness. Despite its importance, longitudinal evidence characterizing post-diarrheal recovery trajectories is sparse. We sought to characterize post-diarrheal nutritional recovery trajectories among children aged 6-35 months who were malnourished at enrollment using data from the Enterics for Global Health (EFGH) *Shigella* Surveillance study (2022-2024). EFGH enrolled children aged 6-35 months presenting with medically-attended diarrhea and followed them at 4 weeks and 3 months post-enrollment. This analysis included children with baseline wasting, stunting, or underweight (z-score < –2) and complete anthropometric follow-up. Latent class mixed-effects models were used to identify distinct post-diarrheal growth trajectories based on changes in anthropometric z-scores over time. Multinomial modified Poisson regression models examined associations between baseline factors and trajectory membership. Among 9,480 enrolled children, 16.5% (n=1,561) were wasted, 22.7% (n=2,155) stunted, and 21.0% (n=1,994) underweight at baseline. Wasting showed greater recovery potential (80.8%) compared with stunting (38.5%) and underweight (40.3%). Recovery was shaped by factors across multiple levels. Clinical severity markers (prolonged diarrhea, dehydration, and hypoxemia) increased the risk of nutritional failure. Age also influenced outcomes: infants were more likely to worsen, whereas older toddlers more often experienced stagnation. Interventions including exclusive breastfeeding, oral rehydration therapy, appropriate antibiotics, and zinc supplementation, improved outcomes, while unimproved sanitation undermined recovery. These findings highlight the need for integrated strategies combining infection control, nutritional rehabilitation, and water, sanitation, and hygiene interventions tailored to the children's developmental stage.

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PRELIMINARY FINDINGS FROM EVALUATION OF THE BIG CATCH-UP IMMUNIZATION RECOVERY PLAN IMPLEMENTATION IN ETHIOPIA, 2025-2026

Kristen Heitzinger[1], Yohannes Lakew Tefera[2], Tatek Bogale[3], Kumie Shiferaw[4], David Schnabel[4], Ciara E. Sugerman[1], Zerihun Girma[5], Abyot Woyessa[6], Amelework Alene[7], Qian An[1], Melkamu Ayalew[2]

[1]U.S. Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Ministry of Health of Ethiopia, Addis Ababa, Ethiopia, [3]African Field Epidemiology Network, Addis Ababa, Ethiopia, [4]U.S. Centers for Disease Control and Prevention, Addis Ababa, Ethiopia, [5]Somali Regional Health Bureau, Jigjiga, Ethiopia, [6]Oromia Regional Health Bureau, Addis Ababa, Ethiopia, [7]Amhara Regional Health Bureau, Bahir Dar, Ethiopia

Ethiopia has an estimated 1.1 million un- and under-immunized children —the fourth highest number globally per the most recent WHO/UNICEF estimates of national immunization coverage—and reported multiple vaccine-preventable disease (VPD) outbreaks in 2025, including measles, polio, and cholera. From 2024-2026, the Ministry of Health of Ethiopia implemented the Big Catch-Up (BCU) initiative to provide un- and under-immunized children aged 12-59 months with missed vaccines to close immunity gaps created during the COVID-19 pandemic. We conducted district level desk reviews and health worker interviews to document lessons learned for long-term catch-up vaccination efforts in six districts across Amhara, Oromia, and Somali Regions. The BCU delivery strategies included fixed and outreach vaccination sessions, periodic intensification of routine immunization, and integration of routine antigens with national polio campaigns. Measuring BCU-specific vaccination coverage was difficult because the rationale for the number of children targeted for catch-up was not consistently documented. However, process indicators suggested that the BCU was generally implemented as planned, as 5 of 6 districts received technical guidance, had the national catch-up vaccination policy, and had information systems capable of capturing age-disaggregated catch-up data. Among health workers interviewed, 77% (287/375) were aware of the national catch-up vaccination policy and 74% (277/375) reported having a catch-up vaccination schedule available. Among health workers who participated in any BCU activities, 15% (19/123) reported that the training was inadequate. This assessment suggests that while key BCU components were in place in most of the selected districts, there were important gaps in health worker training and catch-up monitoring. Addressing these gaps will be critical to improving catch-up vaccination performance and reducing VPD outbreak risk in Ethiopia.

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PLACENTAL FUNCTIONAL IMPACTS OF DIHYDROARTEMISININ-PIPERAQUINE VERSUS SULFADOXINE-PYRIMETHAMINE REVEALED BY SPATIAL TRANSCRIPTOMICS

Stephanie L. Gaw[1], Nida Ozarslan[1], Stephanie Tsoi[1], Christine A. Blauvelt[1], John Ategeka[2], Abel Kakuru[2], Jimmy Kizza[3], Moses R. Kamya[4], Grant Dorsey[1], Philip Rosenthal[5]

[1]Univ of California San Francisco, San Francisco, CA, United States, [2]Infectious Diseases Research Collaboration, Tororo, Uganda, [3]Infectious Diseases Research Collaboration, Busia, Uganda, [4]Makerere University, Kampala, Uganda, [5]Univ of California San Francisco, Mill Valley, CA, United States

Plasmodium falciparum infection during pregnancy is associated with preterm birth, fetal growth restriction, and neonatal mortality. Intermittent preventive treatment of malaria in pregnancy (IPTp) with sulfadoxine–pyrimethamine (SP) is standard policy throughout Africa, and dihydroartemisinin–piperaquine (DP) is under consideration as an alternative for IPTp in areas with SP resistance. A recent trial revealed that IPTp with DP or DP+SP was superior to SP in malaria prevention, but was associated with adverse effects on fetal growth, suggesting placentotoxic effects of DP. However, the molecular impacts of antimalarials on placental function have not been well-studied. To gain insights in this area, we compared the transcriptomes of placentas from pregnancies exposed to DP (n=4) or SP (n=16) with the GeoMx Digital Spatial Profiler. Regions of interest were selected from placental villi and segmented to enrich for syncytiotrophoblasts (panCK), fetal Hofbauer cells (CD68), and fetal endothelial cells (CD31). Differential gene expression and pathway enrichment analysis was conducted on each tissue microregion. In the syncytiotrophoblasts, pathways related to receptor-mediated signaling, ion transport, and immunomodulation were upregulated, suggesting altered environmental sensing and signaling. In contrast, downregulated pathways included protein synthesis/homeostasis and vesicular trafficking, which are classically associated with placental stress response and functional impairment. Fetal Hofbauer cells showed upregulation of cell signaling and extracellular matrix pathways, while ubiquitination and RNA processing/translation were downregulated. Fetal endothelial cells showed upregulation of immune signaling and transcriptional regulation, and downregulation of cell metabolism and stress responses. In all compartments, macroautophagy pathways were significantly downregulated, suggesting

dysregulation of cellular recycling and accelerated placental aging as a global effect of DP. Further studies are needed to understand the placental impact of antimalarials used in pregnancy.

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IDENTIFYING THE CORRELATES OF PROTECTIVE ADAPTIVE IMMUNITY TO CRYPTOSPORIDIOSIS IN BANGLADESHI CHILDREN

Natsuki Watanabe[1], Biplob Hossain[2], Mamun Kabir[2], Brett Moreau[1], Jennie Ma[1], Audrey Brown[3], Zannatun Noor[2], Rashidul Haque[4], William A. Petri[1], Carol A. Gilchrist[5]

[1]University of Virginia, Charlottesville, VA, United States, [2]International Centre for Diarrheal Diseases Research, Dhaka, Bangladesh, [3]Charlottesville, VA, United States, [4]icddr,b, Dhaka, United States, [5]Univ of Virginia, Charlottesville, VA, United States

Cryptosporidiosis is a globally important cause of pediatric diarrhea. In Bangladesh, *Cryptosporidium* infections in children aged 1- 4 years, are an important cause of childhood diarrhea and can exacerbate growth faltering in food-insecure households. Identifying the correlates of protective immunity in these vulnerable children is an important step toward developing an effective vaccine. We are conducting a natural history study of cryptosporidiosis in infants residing in a high-transmission-intensity urban community in Dhaka, Bangladesh, using stool surveillance and twice-weekly household visits to monitor infection and diarrheal incidence. We found that immunity following a first Cryptosporidium infection was incomplete, and reinfections were common. However, in older children with repeated infections, the disease was predominantly subclinical. The incidence of *Cryptosporidium*-attributed diarrhea declined from a peak of 0.19 episodes per child at 1-2 years of age to 0.05 episodes per child at 3-4 years of age. Prior work has shown that total anti-Cp23 and anti-Cp17 IgG is protective in this population. We therefore assessed whether Fc receptor-mediated antibody functions contribute to protection. Given that IgG1 is the predominant subclass mediating these responses, we measured antigen-specific IgG1 levels and FcγRIIIa binding. IgG1 responses to Cp23 and Cp17 variants (Ia and IIa) were associated with protection (Gehan-Breslow-Wilcoxon test; p=0.05, 0.05, 0.07, respectively). Although Fc receptor binding strongly correlated with IgG1 levels (p<0.0001), it was not associated with protection in one-year-old infants. These findings suggest that other IgG1-mediated effector functions may play a more important role.

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GROWTH PATTERNS IN HIV-EXPOSED BUT UNINFECTED CHILDREN: ROLE OF MATERNAL HIV VIREMIA

Nginache Nampota-Nkomba[1], Osward M. Nyirenda[2], Felix Mkandawire[2], Maria Crespo-Llado[3], Cristiana Cairo[1], Melissa J. Gladstone[3], Miriam K. Laufer[1], Andrea G. Buchwald[4]

[1]University of Maryland School of Medicine, Baltimore, MD, United States, [2]Blantyre Malaria Project, Blantyre, Malawi, [3]University of Liverpool, Liverpool, United Kingdom, [4]Liverpool School of Tropical Medicine, Liverpool, United Kingdom

We characterized growth from birth through 5 years of age among children HIV-exposed but uninfected (CHEU) and HIV-unexposed (CHUU) in a prospective cohort in Malawi. CHEU were classified as CHEU-lo (mothers on pre-conception ART, viral load ≤400 copies/mL at pregnancy and delivery) and CHEU-hi (mothers newly diagnosed with HIV, viral load ≥10,000 copies/mL, initiating ART during pregnancy). We enrolled 1229 children (588 CHUU, 528 CHEU-lo, 113 CHEU-hi). Weight and length/height measurements were converted to WHO-standardized indicators: stunting (length/height-for-age Z score, HAZ <–2 SD), underweight (weight-for-age Z score WAZ <-2SD), and wasting (weight-for-height Z score, WHZ <–2SD). Low birthweight (<2500g) was more prevalent among CHEU (12.1% CHEU-lo, 10.6% CHEU-hi) than CHUU (6.9%, p=0.013). In early infancy (<9 months), both CHEU groups showed higher rates of stunting than CHUU, with the greatest burden in CHEU-lo (35.7% vs 28.7% CHEU-hi vs 27.1% CHUU, p=0.016 at 6 weeks and 24.6% vs 23.3% vs 17.6%, p=0.026 at 4 months, respectively). Similar patterns were seen for underweight (10.8% vs 15.6% vs 5.3% , p=<0.001 at 10 weeks and 10% vs 9.3% vs 5.9%, p=0.052 at 4 months, respectively). After 9 months, CHEU-lo and CHUU showed broadly similar growth patterns, while CHEU-hi demonstrated consistently lower rates of stunting and underweight. At 24 months, stunting was 25.6% in CHEU-hi vs 44.4% CHEU-lo and 48.4% CHUU (p=0.016), declining to 5.7% vs 30.0% and 26.7%, respectively by 54 months (p=0.011). Underweight was 0% among CHEU-hi vs 15.4% CHEU-lo and 7.4% CHUU (p=<0.001) at 24 months, and 4.5% vs 14.6% and 7.4% (p=0.025) at 48 months, respectively. Wasting remained low (<6%) throughout, with no consistent differences by group. Despite being born smaller with early growth deficits, CHEU-hi demonstrated substantial catch-up growth after 9 months. CHEU-hi ultimately surpassed both CHEU-lo and CHUU, likely due to confounding from factors like maternal education and socioeconomic status. Additional longitudinal growth trajectory analyses, including confounder adjusted models, will be presented.

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LEAD EXPOSURE IN PREGNANCY AND TRANSPLACENTAL LEAD TRANSFER: A LONGITUDINAL ANALYSIS OF MATERNAL AND UMBILICAL CORD BLOOD LEAD LEVELS IN THE KINTAMPO AREA OF GHANA

Veronica Agyemang[1], Otchere Addai-Mensah[2], David Kwame Dosoo[1], Dennis Adu-Gyasi[1], Dennis Gyasi Konadu[1], Irene Apewe Adjei[1], Lawrence Febir[1], Stephaney Gyaase[1], Eliezer Ofori Odei-Lartey[1], Charlotte Tawiah Agyemang[1], Benedict Sackey[2], Lilian Antwi Boateng[2], Emily Smith[3], Sam Kofi Newton[4], Kwaku Poku Asante[1]

[1]Kintampo Health Research Centre, Research and Development Division, Ghana Health Service, Kintampo, Ghana, [2]Department of Medical Diagnostics, College of Health Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, [3]3Department of Global Health, George Washington University, Washington, D.C, WA, United States, [4]School of Public Health, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

Prenatal lead exposure poses significant public health concerns for both mothers and infants with significant long term adverse outcomes in children. There is, however, paucity of data on maternal and cord blood lead levels in pregnant women in Ghana. This study aimed to assess trimester-specific maternal and umbilical cord blood lead levels (BLLs) and to evaluate transplacental lead transfer in the Kintampo area of Ghana. Maternal blood lead concentrations were measured in each trimester of pregnancy, and cord blood lead levels were measured at delivery for 178 consented mother-infant pairs by inductively coupled plasma-mass spectrometry (NexION 2000, PerkinElmer, USA), following approved standard protocols. Structured questionnaires and standardised measurements were used to collect sociodemographic and anthropometric data. Geometric means (GM) and geometric mean ratios (GMR) with 95% confidence intervals (CI) were calculated to compare trimester-specific changes relative to the first trimester. The mean age of participants was 27.5±5.6 (18 -44) years, with mean gestational age at recruitment being 10.7±2.1 (4.9-13.9) weeks. The GM BLLs increased with increasing trimester: First trimester (1.0 µg/dL; 95% CI: 0.84- 1.12), Second trimester (1.3 µg/dL; 95% CI: 1.15 - 1.41), Third trimester (1.5 µg/dL; 95% CI: 1.29- 1.77) and Cord blood (1.9 µg/dL: 95% CI: 1.69 - 2.18). Cord blood lead levels were 90% higher than first trimester (GMR=1.90, 95% CI: 1.61-2.25, p<0.001) with increases in second (GMR=1.27, 95% CI: 1.08-1.49, p=0.003) and third (GMR=1.49, 95% CI: 1.25-1.76, p<0.001) trimesters. Lead progressively accumulates during pregnancy with substantial transplacental transfer to foetal circulation. Prenatal screening and targeted interventions are recommended to mitigate transplacental lead transfer.

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PREVALENCE AND ASSOCIATED FACTORS OF SEXUAL ACTIVITY AMONG ADOLESCENTS AND YOUTH AGED 13-24 YEARS IN SCHOOL SETTINGS

Hadja Fanta Camara[1], Djiba Diakite[2]

[1]Projet Reach Malaria, Conakry, Guinea, [2]Universite Gamal Nasser, Conakry, Guinea

Adolescent and youth sexuality represents a major public health issue, particularly in school settings where behaviors are formed and experimented with. This study aims to determine the prevalence and associated factors of sexuality among adolescents and young people in schools in Guinea. This is an analysis of data from a cross-sectional survey conducted in 2022 in secondary schools in Guinea. Logistic regression was performed using Stata 14.2. Adjusted odds ratios (AOR) with their 95% confidence intervals (CI) were reported. A total of 11,139 students were surveyed, with a mean age of 17.9 (± 1.8) years. The prevalence of sexuality among adolescents and young people was 42.2% (95% CI: 41.2-43.1). The age group 18-24 years (AOR = 2.63; 95% CI: 2.27-3.04), male sex (AOR = 1.76; 95% CI: 1.53-2.03), married students or those with a partner (AOR = 19.80; 95% CI: 11.99-32.70), Christians and other denominations (AOR = 1.48; 95% CI: 1.27-1.73), the desire to have a child within the next two years (AOR = 1.36; 95% CI: 1.19-1.56), having a partner (AOR = 7.72; 95% CI: 6.66-8.94), and living in the regions of Kankan (AOR = 1.46; 95% CI: 1.17-1.83) and N'zérékoré (AOR = 1.35; 95% CI: 1.13-1.65) were factors associated with sexuality among adolescents and young people in schools. This study provides evidence indicating the need to develop sexual health education within school curricula and other targeted interventions to reduce risky sexual behaviors in school settings.

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REIMAGINING KWASHIORKOR: SOCIOENVIRONMENTAL FACTORS UNDERLYING A CHILD KILLER

Bill H. Kinsey

African Studies Center, Leiden University, Leiden, Netherlands

After more than 90 years, the exact etiology of kwashiorkor is still unknown. Despite the first description of the condition in a specific social and environmental context in the Gold Coast (Ghana), subsequent research has largely focused on dietary and biochemical factors in rather narrow contexts. This paper raises the question of whether poor diet been overstressed as a causative factor in kwashiorkor. It looks at the reasons why some malnourished children, even in the same family, develop marasmus while others develop kwashiorkor, which has a much poorer prognosis. The mortality rate from kwashiorkor can exceed 40 per cent. Kwashiorkor likely arises from a constellation of genetic, biological, social and environmental factors; thus, deepening understanding calls for a broad syndemic approach. Here, based on a very wide-ranging review, together with clinical data from Zimbabwe plus two decades of fieldwork, questions are posed about the relationship between adverse childhood experiences (ACEs) and the onset of kwashiorkor. Specifically, what is the role of the gut-brain axis in mediating the link between ACEs and gastrointestinal conditions? Are ACEs, such as stress or trauma, good predictors of the onset of kwashiorkor? Given the continuing high mortality rates associated with kwashiorkor, and the life-long consequences for survivors, it is past time for attention to be redirected toward its causes.

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GENDER DIFFERENCES AND CROSS-PARTNER ASSOCIATIONS OF FAMILY PLANNING STIGMA AMONG COUPLES IN NORTHERN GHANA

Naa-Korkor R. Allotey[1], Fred Norviemedey[2], Kwasi Torpey[2]

[1]Research and Development Division, Ghana Health Service, Accra, Ghana, [2]School of Public Health, University of Ghana, Accra, Ghana

Family planning (FP) stigma is a key barrier to modern contraceptive use in sub-Saharan Africa, yet evidence on sex-specific predictors and intra-couple dynamics remains scarce. This study examined predictors of FP stigma by sex, assessed cross-partner stigma associations, and evaluated the influence of partners' Theory of Planned Behavior (TPB) constructs on stigma. Cross-sectional data from 508 heterosexual couples in northern Ghana were analyzed. FP stigma was assessed using an adapted version of the Adolescent SRH Stigma Scale and summarized into a composite score. Sex-stratified linear regression identified individual predictors, while actor-partner interdependence models estimated dyadic effects of socio-demographic and TPB constructs on stigma. Significance was set at $p<0.05$ and all statistical analyses were done using STATA /MP version 17. Women reported higher enacted stigma, and overall stigma. Men reported higher self-stigma ($p<0.001$). Poor FP attitudes were strongly associated with higher stigma in men (β=6.44; 95% CI: 5.29-7.59), while poor subjective norms predicted higher stigma in both sexes, with a larger effect in women (β=4.75; 95% CI: 3.16-6.34) than men (β=1.54; 95% CI: 0.68-2.41). Low perceived behavioral control was associated with higher stigma in women (β=3.73; 95% CI: 2.13-5.34), and contraceptive use was associated with lower stigma in men (β=–1.86; 95% CI: –2.99-–0.71). Partner-level analyses revealed that women's stigma was significantly influenced by partner characteristics, including older partner age (β=0.14; 95% CI: 0.02-0.25), poor partner attitudes (β=–2.18; 95% CI: –3.71-–0.66), and low partner perceived behavioral control (β=2.48; 95% CI: 1.00-3.95). In men, only higher partner wealth was significant predictor (β=2.02; 95% CI: 0.17-3.88). No cross-partner spillover in stigma scores was observed. FP stigma differs by sex and is shaped by partner-level social and behavioral factors, particularly among women. Interventions addressing FP stigma in northern Ghana should adopt a couples-based approach in which reproductive decisions are made.

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OPTIMIZING PLACE OF TREATMENT AND ANTIBIOTIC REGIMENS FOR YOUNG INFANTS WITH LOW-MORTALITY-RISK SIGNS OF POSSIBLE SERIOUS BACTERIAL INFECTION IN PAKISTAN

Benazir Baloch

Aga Khan University, Karachi, Pakistan

Study Title: Optimizing Place of Treatment and Antibiotic Regimens for Young Infants with Low-Mortality-Risk Signs of Possible Serious Bacterial Infection in Pakistan, Management of young infants with possible serious bacterial infection (PSBI) typically depends on hospitalization, which may not be feasible in low-resource settings and can increase both financial burden and the risk of hospital-acquired infections. Identifying safe and effective outpatient treatment options for infants with low mortality risk is therefore essential. We conducted a multi-center, open-label, randomized controlled trial in four hospitals of Karachi, Pakistan, to compare standard inpatient care with a simplified outpatient treatment strategy among young infants presenting with low-mortality-risk signs PSBI.Infants aged 0-59 days presenting to secondary and tertiary care hospitals with a single low-risk sign—fever (≥38°C), fast breathing (≥60 breaths per minute in infants younger than 7 days), or severe chest indrawing were eligible for inclusion. Participants were randomly assigned to receive either inpatient treatment with injectable ampicillin plus gentamicin for 7 days, or outpatient treatment consisting of injectable gentamicin for 2 days combined with oral amoxicillin dispersible tablets for 7 days. The primary outcome was poor clinical outcome assessed at day 15, defined as death, progression to critical illness, development of new signs of clinical severe infection (CSI),

or persistence of initial presenting symptoms. Of 3,524 infants screened, 1,566 (44.4%) met eligibility criteria, and 1,125 were randomized (564 inpatient; 561 outpatient). Overall, poor clinical outcomes were infrequent: death occurred in 9 infants (0.8%), progression to critical illness in 6 (0.5%), new CSI signs in 34 (3.0%), and persistence of symptoms in 25 (2.2%). These findings suggest that simplified outpatient treatment may be a safe and feasible alternative for selected low-risk infants, with the potential to reduce hospital admissions, lower healthcare costs, and limit exposure to nosocomial infections in resource-constrained settings.

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STRATEGIC CONSIDERATIONS FOR THE MOST EFFECTIVE AND AFFORDABLE MARKET PRESENTATION OF NEW TREATMENTS AGAINST MALARIA FOR INFANTS AND NEONATES

Sabine Pfeffer[1], Christian Burri[2]

[1]Novartis Pharma AG, Basel, Switzerland, [2]Swiss Tropical & Public Health Inst, Allschwil, Switzerland

Affordability, accurate dosing, and ease of administration of medication are critical to effectively dispense medicines to neonates and small infants. Clinical studies are often performed with small plastic syringes in which e.g. dispersible tablets are dissolved with water and then given to the baby. This procedure has been proven to be feasible, easy to use and robust. Since those plastic syringes might not be readily available in hospitals or health centers in malaria endemic areas, co-packaging of syringes together with the dispersible tablets might be an option. In a study combining a literature search, market research and interviews we analyzed the multifactorial interplay of cost, practice, habit, acceptability, guidance and regulations, and compared co-packaging of mL syringes with e.g. cup feeding, using external spoons and direct application. While co-packaging is not essential, it is feasible from a manufacturing, purchasing, and regulatory perspective, but adds significant complexity and costs. The analysis provided insights that often parents bring their own spoons to the hospital and that cup feeding is an alternative way to administer the medicine to infants in African countries. Cup feeding can be used for babies of all ages and is also safe for premature and many ill babies. International donors are highly price sensitive and prefer simple logistigs, and the added costs of co-packaging might significantly impact future product use. In summary, co-packaging was not recommended. Supplying the product without syringe reduces the price and complexity and simplifies the supply chain and logistics and still enabling accurate dosing and convenience.

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NEGLECTED TROPICAL DISEASES - BURDEN AND BARRIERS IN CONTEXT TO COVID-19

Md Rofiqur Rahman

institute for developing Science & Health initiatives, Dhaka, Bangladesh

Neglected tropical diseases (NTDs) are a broad group of communicable diseases that have proliferated in tropical and subtropical climates, mainly across the developing world, where inadequate sanitation, constant contact with vectors and livestock, and inadequate healthcare services prevail. NTDs are called “neglected” because they have been wiped out of the most developed societies, while they have persisted in the poorest and most marginalized ones. Due to the COVID-19 pandemic, many key neglected tropical disease (NTD) activities have been postponed. This hindrance comes at a time when the NTDs are progressing towards their ambitious goals for 2030. Several NTDs, namely gambiense sleeping sickness, lymphatic filariasis, onchocerciasis, schistosomiasis, soil-transmitted helminthiases (STH), trachoma, and visceral leishmaniasis, are on the tracks and progressing towards the goal achievements. But the impact of COVID shows that the disruption in execution and barriers in access could vary across the diseases. Programs might face a risk of resurgence, which will be fastest in high-transmission areas. The case-finding diseases are likely to have fewer cases being detected but may face an increasing underlying rate of new infections. However, once programs are able to resume, there are ways to mitigate the impact and accelerate progress towards the 2030 goals. The COVID-19 pandemic had significant impact on the fight against NTDs, however, it is paramount that already neglected tropical diseases do not become more neglected. Health messaging through the bill boards, radio, TV slots will not only reduce the risk of COVID-19 community transmission but also enhance adherence to the necessary behavior change. The countries also need to come up with community information across the borders to train the community health staff on disease control measures. The fact that COVID 19 now disproportionately affects the economies of the world’s most endemic NTD nations is an ominous sign and its impact could derail otherwise promising control and elimination efforts.

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UNDERSTANDING AT-RISK COUNTRY PERSPECTIVES ON NIPAH VACCINE AND MONOCLONAL ANTIBODY USE TO INFORM DEMAND FORECASTING

Katelyn A. Dinkel[1], Anika Ruisch[2], Ishani Mathur[2], Rebakaone Bow[1], Peter Windus[1], Justice Nonvignon[2], Damian Walker[2], Andrew A. Torkelson[1]

[1]Linksbridge SPC, Seattle, WA, United States, [2]Management Sciences for Health, Arlington, VA, United States

Nipah virus (NiV) is a zoonotic pathogen that causes episodic outbreaks of severe disease in humans. NiV was designated as a priority disease/pathogen by the World Health Organization and the Coalition for Epidemic Preparedness Innovations. These organizations are supporting research into medical countermeasures such as vaccines and monoclonal antibodies (mAbs). Despite being a priority, little is understood about how at-risk countries, particularly low- and middle-income, intend to use vaccines and mAbs. Our work addresses this gap through interviews and demand forecasting for 10 countries: Australia, Bangladesh, Cambodia, India, Indonesia, Malaysia, the Philippines, Singapore, Thailand, and Vietnam. Interviews were conducted with country- and global-level stakeholders to gather insights on regional sentiment, financing mechanisms, and affordability. Profiles were developed for each country that summarized perspectives on perceived risk of NiV outbreaks and countermeasure preferences. Demand was estimated by integrating findings into models of likely country responses to outbreak scenarios across three magnitudes: expected, amplified, and extended. In country interviews, the degree of NiV preparedness and awareness varied, but disease burden, financial constraints, and intervention familiarity were the most important considerations for policymakers. Global demand started ~800-4,500 doses in reactive responses to expected annual outbreaks. This demand increased with outbreak size in subsequent scenarios after country responses expanded to include preventive campaigns for at-risk populations. This study highlighted existing perspectives on Nipah disease and countermeasures, and enumerated demand levels across three outbreak scenarios. For expected outbreaks, annual demand is low and episodic, posing a challenge to sustainable production. To mitigate this challenge, it is recommended that an international stockpile be maintained and that the factors identified as limits to country demand—intervention affordability and familiarity—be addressed to help support adoption in the event of an outbreak.

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DESIGNING AND COSTING A CROSS-BORDER DISEASE SURVEILLANCE SYSTEM IN THE GAMBIA AND SENEGAL: A SYSTEMS THINKING APPROACH

Haddijatou Allen[1], Hilary Bower[2], Sergio Torres Rueda[1], Effua Usuf[3], Melisa Martinez-Alvarez[1], Fiammetta Bozzani[1]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]UK Public Health Rapid Support Team, London, United Kingdom, [3]MRC Unit The Gambia at LSHTM, Fajara, Gambia

Disease surveillance systems across sub-Saharan Africa remain under-resourced and fragmented. In West Africa, where over 80% of migration

is intraregional, cross-border disease spread presents challenges no national system can address alone. The Gambia and Senegal share a 740 km border with high cross-border health-seeking, yet their Integrated Disease Surveillance and Response (IDSR) systems operate with minimal coordination. Building on a qualitative IDSR evaluation identifying fragile informal coordination, absent cross-border information sharing and inadequate border health services as key barriers, this study investigates constraints and enablers to cross-border surveillance (CBS), identifies strategies and cost drivers. A mixed-methods study was conducted across both countries. Interviews with 39 purposively sampled stakeholders were analysed using causal loop diagrams (CLDs) to map feedback structures and leverage points across seven domains; prospective costing adopted a government-funded perspective. CBS relies on informal relationships, reflecting absent infrastructure for coordinated detection and response. CLD analysis identified reinforcing loops perpetuating fragmentation: governance constraints prevent district teams acting without central approval, delaying outbreak response. Donor dependency has prevented domestic budget line development; recent withdrawal has exposed this vulnerability, with detection gaps disproportionately affecting mobile and marginalised populations. Costing revealed Senegal bears a disproportionate CBS cost share, reflecting stronger baseline infrastructure. High-leverage interventions include: formalising CBS agreements, earmarking domestic budget lines, integrating DHIS2 systems and training community surveillance volunteers, with programmatic actions enabling service-level gains. Systems thinking identified governance investment as the primary binding constraint, required before technical inputs can strengthen CBS. The two-tier strategy offers a roadmap for governments establishing equitable CBS systems across West Africa.

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DISEASE WITHOUT BORDERS: MODELING EPIDEMIOLOGICAL TRENDS OF INFECTIOUS DISEASES IMPACTED BY CONFLICT AND MIGRATION

Lucy R. Lawrence[1], Michael E. DeWitt[1], John W. Sanders[2]
[1]Wake Forest University School of Medicine, Winston Salem, NC, United States, [2]Wake Forest University, Winston-Salem, NC, United States

War, persecution, and economic crises have driven the movement of people and the diseases they carry for centuries. The displacement of refugees globally has posed unique, shifting challenges for public health officials and the healthcare systems of receiving countries. Not only does mass migration introduce new populations to each other, but the conflicts underlying these migrations further erode already established public health infrastructure. And these infectious diseases are not contained to refugee camps, but also seep into local communities. Furthermore, infectious diseases are not static, but are rather impacted by many factors that make it challenging to predict when, and which, diseases will crop up. We examined publicly reported public health surveillance data from the Lebanese Ministry of Public Health to better understand the frequency and periodicity of reportable communicable diseases in Lebanon during the Syrian civil war. To assess the presence of temporal associations of disease incidence we conducted a cross-correlation analysis, demonstrating the estimated number of cases of reportable diseases in Lebanon caused by the Syrian civil war. Our cross correlation analysis found that leishmaniasis, measles, and brucellosis had a positive correlation with a high negative lag, demonstrating a peak in the Syrian refugee community corresponding to a similar peak in the Lebanese population months later. This suggests a significant and measurable effect of forced migration on domestic disease transmission. While our analysis establishes the ripple effect of refugees, it also offers potential solutions; improving sanitation standards in refugee camps, routine health maintenance, and investing in disease prevention like chemoprophylaxis benefits the host country as much as the refugee community. By better understanding the patterns that undergird disease spread after a mass migration, we can better anticipate which communicable pathogens will take hold first and can work to minimize the burden of disease for both foreign and host populations.

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MULTI-PATHOGEN SEROSURVEILLANCE OF VACCINE PREVENTABLE AND NEGLECTED TROPICAL DISEASES TO IDENTIFY PRIORITY POPULATIONS FOR TARGETED PUBLIC HEALTH ACTION

Selina Rachael Ward[1], Harriet Lawford[2], Benn Sartorius[1], Helen Mayfield[1], Filipina A.L Sam[3], Sarah Sheridan[4], Robert Thomsen[5], Satupaitea Viali[6], Stefanie Vaccher[7], Leanne J. Robinson[7], Fiona Angrisano[7], Colleen L. Lau[1]
[1]University of Queensland, Brisbane, Australia, [2]The University of Queensland, Nundah, Australia, [3]National University of Samoa, Apia, Samoa, [4]National Centre for Immunisation Research and Surveillance, Sydney, Australia, [5]Samoa Ministry of Health, Apia, Samoa, [6]Oceania University of Medicine, Apia, Samoa, [7]Burnet Institute, Melbourne, Australia

Serosurveillance estimates can reflect antibodies (Ab) acquired through vaccination or current and/or past infection. Compared to a siloed approach, multi-pathogen serosurveillance enables identification of vulnerable sub-populations with immunity gaps to vaccine preventable disease (VPD) and concurrent burden of neglected tropical disease (NTD). This study aimed to identify temporal seroprevalence of selected VPDs/NTDs in Samoa to inform targeted public health action, where lymphatic filariasis (LF) remains endemic despite ongoing elimination efforts. Dried blood spots were collected from 4 repeated community-based surveys in 8 primary sampling units (PSU) in 2018, 2019, 2023 and 2024. Multiplex bead assays were used to detect Abs against antigens (Ag) for VPDs (diphtheria, measles, rubella, tetanus) and NTDs (LF [Wb123 or Bm14], yaws [Rp17 and TmpA], and trachoma [Pgp3]). Seroprevalence estimates were adjusted for sampling design and standardised for age and sex. Of the 2871 participants (551 in 2018; 625 in 2019; 598 in 2023; 1097 in 2024) included, marked variation was seen across PSU, pathogens and time. Between 2018 and 2024, mean PSU-level seroprevalence to measles increased from 71.1 to 96.6 ($p<0.001$), while seroprevalence to diphtheria declined steadily from 89.2 to 65.8% ($p<0.001$). In contrast, for all PSUs, seroprevalence remained low for yaws (<3.7% in 2018; <13.8% in 2024 [$p=0.001$]) and trachoma (mean all years 8.6%), while seroprevalence to LF (either Ab) declined from 53.5% (2018) to 24.0% (2024; $p<0.001$). Importantly, this study identified 15 individuals (mean=17 yrs) seronegative to all VPDs in 2018 and 2019; of which 8 (mean=15 yrs) were also seropositive to at least one NTD. Identification of vulnerable sub-populations with concurrent seronegativity to VPDs and seropositivity to NTDs underscores the potential role of multi-pathogen serosurveillance in directing public health interventions to those at greatest risk of multiple infectious diseases. Identification of temporal changes offers a valuable tool for targeting and refining LF elimination efforts and measuring the impact of interventions.

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MAGNETIC HYDROGEL PARTICLE-BASED MICROBE CONCENTRATION ENABLES SCALABLE DETECTION OF *MYCOBACTERIUM* ACROSS CLINICAL AND WASTEWATER MATRICES

Ben Lepene, Patrick Andersen, Anurag Patnaik, Sayed Mosavi
Ceres Nanosciences, Inc., Manassas, VA, United States

Tuberculosis (TB) remains one of the most consequential infectious diseases, imposing substantial health, social, and economic burdens worldwide, particularly in low- and middle-income countries. Molecular diagnostics such as quantitative PCR (qPCR) and digital PCR (dPCR) offer faster results than culture but are often limited by inefficient sample preparation and inhibitory effects in complex matrices such as sputum. Wastewater-based epidemiology provides a complementary, population-level surveillance strategy for *Mycobacterium*; however, scalable concentration workflows applicable across clinical and environmental matrices remain limited. We evaluated Nanotrap Microbiome Particles, functionalized magnetic hydrogel particles, for capture and concentration of *M. smegmatis* and *M. abscessus* as surrogates for *M. tuberculosis*.

Sputum (1-4 mL) and wastewater (10 mL) samples were processed using Nanotrap Microbiome B Particles, followed by a magnetic bead-based nucleic acid extraction on an automated magnetic platform. Performance was compared to centrifugation-based workflows. Increasing sputum input from 1 to 4 mL improved detection sensitivity across tested concentrations, corresponding to an approximate 2-12 fold increase in signal by qPCR and a 3-12 fold increase in absolute quantification by dPCR. The Nanotrap Particle workflow demonstrated increased recovery of *M. smegmatis* from diagnostic remnant sputum while reducing processing time relative to centrifugation-based methods. In wastewater, the Nanotrap Particle workflow demonstrated higher target recovery than a centrifugation-based method, including 2 orders of magnitude higher signal at higher concentrations and consistent detection at lower concentrations where centrifugation-based processing yielded no detectable signal. These findings support a magnetic hydrogel-based workflow as a scalable, centrifugation-free approach for microbial detection across clinical and environmental matrices, with potential utility for TB diagnostics and environmental surveillance.

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A PATHOGEN- AND VECTOR-AGNOSTIC WORKFLOW FOR VECTOR-BORNE DISEASE SURVEILLANCE

Niel Infante[1], Jennifer Bushing[1], Mary Katherine Lynn[2], Melissa S. Nolan[2], Brandon D. Hollingsworth[2], **Shaun T. Cross**[1]

[1]University of Nebraska Medical Center, Omaha, NE, United States, [2]University of South Carolina, Columbia, SC, United States

Vector-borne disease (VBD) incidence is rising rapidly in the United States and globally. Military personnel are at elevated risk due to prolonged outdoor exposure and deployments to VBD endemic regions, particularly in areas with damaged or limited infrastructure such as conflict and disaster zones. This operational vulnerability highlights the need for early outbreak detection systems that function reliably in low resource environments and can identify a wide range of known and (re)emerging pathogens. To address this capability gap, we developed a vector- and pathogen-agnostic workflow combining a hybrid capture sequencing platform with model informed adaptive surveillance to provide near real-time risk maps. Our bioinformatic pipeline: (i) generates probe sequences from user defined targets, (ii) performs quality control filtering to remove off target or redundant probe candidates, and (iii) conducts in silico modeling to predict capture performance and estimate sequencing depth required for desired genome coverage. This data is then used to inform an ensemble model for predicting local areas of potential risk and, in the case of detected pathogens, likely transmission zones. Here, we present preliminary results from our bioinformatic workflow. We aggregated all publicly available arbovirus genomes (>4,000 representative sequences) and gene target references for 85 bacterial and 48 parasitic pathogens. These inputs were used to design approximately 132,000 biotinylated probes, which are produced inhouse to reduce supply-chain burden and cold-chain dependency. We evaluated the probe set against viral, bacterial, and parasitic vector-borne pathogens across multiple arthropod vectors. Performance was comparable to commercial probe sets but at a substantially lower cost. Collectively, these results demonstrate a robust, scalable, and cost-effective platform for pan-VBD detection and diagnostics. Future work will focus on operational field integration into our computational workflow to enhance real-time exposure-risk prediction and inform targeted preventive interventions for military personnel.

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FROM EVIDENCE TO STRATEGY: DEVELOPING CÔTE D'IVOIRE'S NATIONAL STRATEGIC PLAN FOR ARBOVIRUS PREPAREDNESS AND RESPONSE

Poe Poe Aung[1], Lucien Yao Konan[2], Constant G.N Gbalegba[3], Rosine Z Wolie[4], Samuel Kweku Dadzie[5], Tarekegn A. Abeku[6], Leo Braack[1], Sian E. Clarke[7]

[1]Malaria Consortium, Bangkok, Thailand, [2]National Institute of Public Hygiene, Ministry of Health, Abidjan, Côte D'Ivoire, [3]National Malaria Control Program, Ministry of Health, Abidjan, Côte D'Ivoire, [4]Vector Control Product Evaluation Centre (VCPEC), Institut Pierre Richet (IPR), Abidjan, Côte D'Ivoire, [5]Noguchi Memorial Institute for Medical Research, Accra, Ghana, [6]Malaria Consortium, London, United Kingdom, [7]London Sch of Hygiene & Tropical Medicine, London, United Kingdom

A 2024 self-assessment of arbovirus outbreak preparedness in Burkina Faso, Cameroon, Côte d'Ivoire, and Tanzania revealed critical gaps and absence of comprehensive national strategic plan (NSP), despite the existence of related policy documents. In response, the Resilience Against Future Threats (RAFT) consortium supported Côte d'Ivoire as a model in developing a NSP on Arboviruses that aligns with WHO standards. Using an Implementation Research approach to ensure local ownership and uptake, the process included country's self-assessment comprising stakeholder interviews and self-assessment surveys completed by representatives from relevant departments in the Ministry of Health (MOH). A series of workshops with the national stakeholders, supported by a scoping review of policy documents and literature review from 2019 to 2024. The Core Writing Team, composed of four members, including an external consultant, the Ministry of Health, and research institutions, led the development of the NSP, supported by a Technical Working Group of 11 members from various institutions and three subwriting teams comprising 20 departmental representatives, with the Advisory Team providing technical review. Five strategic interventions in the NSP include reducing the risk of arboviral diseases transmitted by Aedes mosquitoes within the population through vaccination and prevention measures, enhancing surveillance of Aedes-borne viruses and prediction of outbreak risks, enhancing vector control strategies for the management and containment of epidemics, promoting state-of-the-art approaches in risk prediction, response and control and fostering national multisectoral coordination and collaboration among stakeholders and partners for preparedness, response and resilience to *Aedes*-borne arboviral diseases. This case study offers a practical, locally owned framework for improving surveillance and response and provides a replicable model for other countries seeking to close critical gaps in arbovirus readiness.

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VETERINARY PRESCRIPTIONS AS A ONE HEALTH SURVEILLANCE SIGNAL FOR ZOONOTIC LEISHMANIASIS IN TUSCANY, ITALY: IMPLICATIONS FOR PUBLIC HEALTH

Claudia Cosma[1], Ezio Ferroglio[2], Andrea Guida[3], Carla Maia[4], Benjamin Rader[5], Margherita Cacini[6], Barbara Rossi[6], Giovann Bianco[6], Enrico Loretti[6], Bonanni Paolo[3], Chiara Lorini[3], Guglielmo Bonaccorsi[3], Marco Del Riccio[3], Martina Benedetti[7], Antonino Barone[7], Nicolas Ray[1]

[1]University of Geneva, Geneva, Switzerland, [2]University of Turin, Turin, Italy, [3]University of Florence, Florence, Italy, [4]Universidade Nova de Lisboa, Lisbon, Portugal, [5]Harvard Medical School, Boston, MA, United States, [6]Tuscany Regional Government, Florence, Italy, [7]Experimental Zooprophylactic Institute of Lazio and Tuscany, Florence, Italy

In endemic settings, canine leishmaniasis is not only a veterinary concern but also a public health challenge. In Tuscany, Italy, where zoonotic leishmaniasis is sustained by *Leishmania infantum*, surveillance is almost absent in dogs because canine infection is not subject to mandatory notification, despite dogs being central to the transmission cycle. We assessed whether veterinary prescriptions could provide a spatial and temporal signal of clinically managed canine leishmaniasis in Tuscany for One Health surveillance of zoonotic leishmaniasis. This retrospective ecological study used electronic veterinary prescription data from April 2019 to December 2025. Municipality-level indicators of antileishmanial drug packages and vaccine doses were derived and standardized per 1,000 registered dogs. Temporal trends, spatial patterning, and the association between therapeutic and preventive prescribing were assessed. A total of 10,403 antileishmanial drug packages and 12,061 vaccine doses were recorded. Prescribing remained strongly treatment-oriented, with meglumine antimoniate accounting for most drug use. Drug prescribing increased over time and showed recurrent spatial concentration in southern Tuscany. Vaccine prescribing also increased but had weaker

and less persistent spatial clustering. The association between vaccine and drug use was modest, likely due to low vaccination coverage and less spatially targeted preventive prescribing (Spearman ρ≈0.08-0.37). Veterinary prescriptions capture clinically treated canine disease, not infection burden. However, in the absence of mandatory notification, they provide a reproducible complement to conventional surveillance, helping identify where clinically managed burden accumulates. Recurrent canine therapeutic concentration in Grosseto-Maremma area overlaps with a major focus in Tuscany where human cases have also been reported. While these data cannot replace routine human, veterinary, or entomological surveillance, they can strengthen integrated surveillance by highlighting priority areas for earlier and more targeted public health action.

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NUTRITIONAL APPROACHES TO PRESERVING *ANOPHELES GAMBIAE* EGGS AND ADULTS

Jia Yue Zhao[1], Emma Camacho[2], Arturo Casadevall[2]

[1]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [2]Johns Hopkins Univ, Baltimore, MD, United States

Sustainable implementation of next-generation vector control methods requires mass rearing, which remains costly, labour-intensive, sensitive to environmental factors and to genetic mutation. This project explores the possibility of inducing a diapause-like state in *Anopheles gambiae* eggs and adults to counter unideal rearing and storage conditions. By reducing the burden of laboratory colony management and improving mosquito resilience, strategies such as gene drive can be more easily implemented by reducing losses during storage and transport, maintaining a stable population for field release, and lowering rearing and environmental maintenance costs. This approach is inspired by resistance strategies in other mosquito species, as well as melanin protection in fungal systems. In *Aedes* and *Culex* mosquitoes, diapause can occur in eggs and mated female adults, respectively. The close evolutionary relationship of the mosquito species suggests that *Anopheles* mosquitoes may retain some degree of behavioural plasticity to 'hibernate'. In *Cryptococcus neoformans*, melanin is deposited in association with lipids and polysaccharides, indicating that environmental stress tolerance depends on an appropriate ratio of these components. As it has been previously established that melanin offers thermotolerance in *Anopheles* mosquitoes, it can be hypothesized that similar melanin principles may contribute to increasing the resilience of mosquitoes. The two nutritional compounds, chitin precursor GlcNAc and melanin precursor L-DOPA, will be supplied maternally, as it has been demonstrated that the diet and gene expression of female mosquitoes can influence the eggs they lay. Along with enhancing melanization, L-DOPA may alter lipid mobilization, potentially through a maternal pathway, involving the gene *tryglyceride lipase 2*. GlcNAc may support chitin deposition in the serosal cuticle of eggshells to reduce desiccation. These effects will be evaluated through eggshell chitin and lipid content, maternal *TL2* expression and adult survival under adverse conditions.

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THE PRESENCE OF THE *WAND STRAIN* OF *WOLBACHIA* IS CORRELATED WITH LOWER LEVELS OF *PLASMODIUM* SPOROZOITES AND A LESS DIVERSE MICROBIOME IN WILD *ANOPHELES DEMEILLONI* MOSQUITO CEPHALOTHORAXES

Seynabou Sougoufara[1], Janvier Bandibabone[2], Laura Chatteley[1], Isabel Polly Claire Hughes[1], Carolina Molina Taberes[1], Jade Ball[1], Thomas Palliaser[1], Vishaal Dhokiya[3], Eva Heinz[4], Grant Hughes[3], Thomas Walker[1]

[1]University of Warwick, Coventry, United Kingdom, [2]Laboratoire d'entomologie médicale et parasitologie, Centre de Recherche en Sciences, Naturelles (CRSN/LWIRO), Sud-Kivu Democratic Republic of Congo., Democratic Republic of the Congo, [3]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [4]University of Strathclyde, Glasgow, United Kingdom

The increasing resistance of malaria vectors against insecticides is an urgent concern for disease control and novel vector control strategies are needed. *Wolbachia* are endosymbiotic bacteria that can invade mosquito populations and reduce transmission of human pathogens. *Wolbachia* strains in wild *Anopheles* species are rare, with only two known examples of genuine symbiosis; *An. moucheti* with *w*AnM and *An. demeilloni* with *w*AnD. In this study, we set out to determine if there was a correlation between *w*AnD in different *An. demeilloni* mosquito body parts, the sporozoites in cephalothoraxes, and the overall mosquito microbiome. We undertook a combination of qPCR and *16S rRNA* amplicon sequencing in the body parts of *An. demeilloni* collected in 2021/2024 from the DRC to determine *Wolbachia* prevalence and densities within the wider mosquito microbiome and to screen *Plasmodium falciparum* sporozoites in cephalothoraxes. *Wolbachia* prevalence rates were significantly higher in abdomens compared to cephalothoraxes and density was also significantly higher in abdomens ($P<0.0001$). The overall sporozoite prevalence was 1.3% which was not significantly different between *Wolbachia*-positive and *Wolbachia*-negative cephalothoraxes ($P=0.3630$) despite *Pl. falciparum* only detected in *Wolbachia*-negative cephalothoraxes. However, *Wolbachia*-positive abdomens were significantly associated with a lower sporozoites rate compared to *Wolbachia*-negative abdomens ($P=0.0329$). *16S rRNA* amplicon sequencing revealed no significant difference in alpha/beta diversities between abdomens and cephalothoraxes but the cephalothorax microbiome composition between *Wolbachia*-positive and *Wolbachia*-negative was significantly different ($P<0.05$). Our findings indicate a significant effect of the *w*AnD strain on both the mosquito cephalothorax microbiome and potentially the ability of sporozoites to reach the salivary glands. Further studies are needed to determine the mechanisms in which the *w*AnD strain interacts with *Plasmodium* sporozoites and if this strain could be used for malaria biocontrol through transinfection of major malaria vectors.

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FROM COPULATION TO FERTILIZATION: UNRAVELING MOSQUITO SPERM BIOLOGY FOR NEXT GENERATION VECTOR CONTROL

Laura C. Harrington[1], Claudia A.S. Wyer[1], Jake W. Angelico[2], Mariana F. Wolfner[1]

[1]Cornell University, Ithaca, NY, United States, [2]Ithaca, NY, United States

As genetic control strategies for mosquito vectors become increasingly feasible, identifying alternate physiological targets to enhance outcomes or preempt resistance is of paramount importance. Despite offering significant potential for highly effective reproductive control, mosquito sperm biology remains underexplored. During copulation, sperm is transferred to the female alongside a complex proteinaceous cocktail of molecules that together are essential for reproductive success. The sperm navigates from the female bursa to the spermathecae, specialized organs that maintain sperm viability throughout the entire lifespan of the female. This presentation synthesizes current knowledge regarding the intricate trajectory of mosquito sperm from copulation to egg fertilization in the arbovirus vector, Aedes aegypti. We highlight our recent advancements in understanding sperm biology and storage, as well as the complex interplay of male and female derived molecules that facilitate this delicate reproductive process. By unraveling these fundamental biological interactions, we underscore the potential of sperm biology as a promising frontier for the development of next generation vector control interventions.

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ANOPHELES SALIVARY ANTIGEN REACTIVITY AS A QUANTITATIVE BIOMARKER FOR MOSQUITO EXPOSURE IN INTERVENTIONAL STUDIES

Haley Abernathy[1], Dana Giandomenico[2], Anne Starling[1], Berlin L. Londono[3], Jonathan J. Juliano[4], Ross M. Boyce[5]

[1]University of North Carolina at Chapel Hill, Chapel Hill, NC, United States, [2]University of North Carolina at Chapel Hill, Pittsboro, NC, United States, [3]Tulane University, New Orleans, LA, United States, [4]Univ of North Carolina, Chapel Hill, NC, United States, [5]University of North Carolina Chapel Hill, Chapel Hill, NC, United States

The gSG6-P1 salivary peptide of *Anopheles* mosquitoes has emerged as an important biomarker of human-vector exposure. It is distinct from traditional measures (e.g., disease incidence) in that it does not rely on pathogen transmission and development of disease. Current evidence suggests that it correlates well with *Anopheles* exposure, specifically *Anopheles gambiae* and *Anopheles funestus,* the primary malaria vectors in East Africa. Since its discovery, anti-gSG6-P1 antibodies have been used across a range of studies to characterize seasonal biting behavior, geographic heterogeneity in malaria transmission, and measure early childhood exposure. However, its use in a short-term longitudinal setting as a proxy for vector exposure and as a measure of intervention effectiveness has not been thoroughly investigated. Our study investigates the role of *Anopheles* salivary antigen in interventional studies by leveraging data and dried blood spots from a randomized control trial in Uganda (Boyce et al., NEJM, 2025) in which the treatment and control arm experienced significantly difference clinical malaria incidence rates among children aged 6-18 months old after the implementation of permethrin-treated baby wraps (2.14 (95% CI 1.73-2.62) vs. 0.73 (95% CI 0.51-1.02)). Our overarching hypothesis is that the treated wraps effectively deter mosquito landing and biting, which should result in higher levels of anti-gSG6-P1 antibodies among the control as compared to intervention arms. Dried blood spot extracts are currently undergoing enzyme linked immunosorbent assay (ELISA) testing with a scheduled completion date of April 17th, 2026. We will present the baseline levels of anti-gSG6-P1 IgG antibodies between children with and without *P. falciparum* parasitemia at enrollment and compare longitudinal gSG6-P1 antibody response between children in the control and treated arms of the parent trial.

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CHARACTERIZATION OF SEX-SPECIFIC PROTEINS BOUND TO SPERM IN THE DENGUE VECTOR *AEDES AEGYPTI*

Jake W. Angelico[1], Irene Alexandra Amaro[1], Sylvie Pitcher[1], Claudia A.S Wyer[1], David Andrew Ellis[2], Steve Dorus[3], Mariana F. Wolfner[1], Laura C. Harrington[1]

[1]Cornell University, Ithaca, NY, United States, [2]University College London, London, United Kingdom, [3]Syracuse University, Syracuse, NY, United States

Female *Aedes aegypti* typically mate only once, storing sperm for egg fertilization throughout their entire lifespan. However, the specific molecular mechanisms governing sperm viability, motility, and long-term storage post ejaculation remain poorly understood. While male-derived proteins are known to perform critical roles in these functions, and to bind to sperm in *Drosophila melanogaster,* analogous processes in mosquito vectors require further elucidation. Furthermore, in *D. melanogaster* female-derived proteins also bind to sperm post-mating and during prolonged storage, and have been suggested to act to maintain sperm viability and function. Here, we investigated the dynamic binding of sex-specific proteins to *Ae. aegypti* sperm and the temporal changes of these protein profiles throughout the sperm-storage period. Utilizing reciprocal reverse label proteomics, we identified sex-specific proteins bound to sperm immediately after copulation and across several time points during storage. Our results reveal distinct temporally dynamic profiles of both male and female-derived proteins bound to sperm. Our findings highlight novel candidate proteins for regulating sperm viability and function in these mosquitoes, and that could be new targets for interventions aimed at reducing vector populations in endemic regions.

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ROLE OF *ANOPHELES GAMBIAE* MESH IN THE MOSQUITO MIDGUT HOMEOSTASIS

Yiwen Zhang[1], Alberto Medina[1], Emma Camacho[2], Arturo Casadevall[1]

[1]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [2]Johns Hopkins Univ, Baltimore, MD, United States

Malaria transmission depends on successful *Plasmodium* development within the *Anopheles* mosquito midgut, where epithelial barriers and immune responses regulate parasite survival. In this study, we characterized *Anopheles gambiae* MESH (*Ag*MESH), a conserved mucin-like protein, and investigated its role in midgut barrier function and parasite development. We observed that the *Ag*MESH gene expression was significantly upregulated within 12 h post blood meal (pbm), coinciding with Type 1 peritrophic matrix (PM) formation, and was predominantly localized to the midgut. *Ag*MESH gene silencing in *A. gambiae* mosquitoes was associated with compromised integrity of the PM, suppressed synthesis of dityrosine network (DTN) due to heme peroxidase (IMPer) inability in catalyzing protein crosslinking, and reduced *Plasmodium* parasite infection. Meanwhile, culturable midgut bacterial load was reduced following *Ag*MESH gene silencing, indicating the possibility that the *Plasmodium* parasite viability in the midgut is reduced by activated immune defenses primarily against bacteria boosted by blood intake. Together, our findings support a model in which *Ag*MESH functions as a structural and regulatory component of the midgut barrier, coordinating PM integrity, microbiota homeostasis, and immune modulation to influence malaria parasite survival. Given the high conservation of *Ag*MESH among disease-transmitting insect vectors, future analysis targeting *Ag*MESH may provide a novel strategy for reducing mosquito vector competence and blocking disease transmission.

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MICROBIOTA COMPOSITION DIFFERS BETWEEN *PLASMODIUM*-INFECTED AND NON-INFECTED *ANOPHELES GAMBIAE S.L.* IN MALI

Salif Thiam[1], Moussa Keita[1], Alvaro Molina-Cruz[2], Nafomon SOGOBA[1], Abassana Ine dit Lazarre Yalcouye[1], Carolina V. Barillas-Mury[3]

[1]Infectious Diseases, Medical Entomology, and Training Center, Bamako, Mali, [2]Laboratory of Malaria and Vector Research/NIAID/NIH, Rockville, MD, United States, [3]National Institutes of Health, Rockville, MD, United States

Mosquito microbiota play an important role in vector competence and *Plasmodium* development. However, data on microbiota associated with natural *Plasmodium* infection in West African Anopheles populations remain limited. This study investigated bacterial communities in field-collected *Anopheles gambiae s.l.* according to infection status and temporal variation. Mosquitoes were collected in Makono (Koulikoro, Mali) from October to December 2018. *P. falciparum* infection was determined using a circumsporozoite protein ELISA on mosquito thorax homogenates. Thorax and abdomen microbiota were characterized using high-throughput 16S rRNA gene sequencing. Alpha, beta diversity, and linear discriminant analysis effect size (LEfSe) were used to compare microbial communities within and between infected and non-infected mosquitoes. Thorax microbiota showed significantly lower diversity than abdomen microbiota, with *Proteobacteria*, including *Pseudomonas*, being the predominant phylum. Alpha-diversity of abdomen samples did not differ by infection status. However, beta diversity analysis revealed significant differences in microbial community structure between infected and non-infected abdomens. Genera including *Enterobacter, Azotobacter, and Erwinia* were enriched in infected mosquitoes, whereas *Sphingomonas, Rhodococcus, and Escherichia-Shigella* were more abundant in non-infected mosquitoes. Temporal variation was observed, with *Enterobacter* associated with

infected mosquitoes in December, while *Chryseobacterium* was enriched in non-infected mosquitoes in November and December. Distinct microbial signatures are associated with natural *Plasmodium* infection in *Anopheles gambiae s.l*. mosquitoes and highlight temporal variation in mosquito gut microbiota. These findings suggest that microbiota may influence mosquito susceptibility and highlight potential targets for microbiome-based vector control strategies.

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DIETARY L-DOPA MODULATES *ANOPHELES* MOSQUITO MICROBIOME AND REDUCES MALARIA TRANSMISSION POTENTIAL

Emma Camacho, Arturo Casadevall

Johns Hopkins University, Baltimore, MD, United States

Malaria remains a major global health challenge, exacerbated by insecticide resistance and climate-driven expansion of mosquito habitats. These limitations underscore the need for sustainable, non-insecticidal strategies to reduce vectorial capacity. We test whether dietary L-3,4-dihydroxyphenylalanine (L-DOPA), a plant-derived melanin precursor found in nectar, can modulate mosquito physiology and microbiome composition to reduce *Plasmodium falciparum* transmission. We hypothesize that L-DOPA reduces *Anopheles gambiae* vectorial capacity through microbiome-dependent mechanisms that enhance immune activation and alter cuticle composition. This is supported by L-DOPA's dual role as a metabolic substrate and redox-active compound influencing host melanization and microbial community structure. Adult female mosquitoes were maintained on sucrose diets ± L-DOPA, and midgut microbiota were analyzed by 16S rRNA sequencing. Microbiome analyses reveal increased bacterial diversity and a shift from *Elizabethkingia*-dominated communities to more diverse assemblages enriched in *Serratia* and *Chryseobacterium*, taxa linked to immune activation and detoxification. Ongoing studies aim to establish causality via microbiome depletion and recolonization, and to assess fungal contributions. Notably, L-DOPA exhibits a hormetic dose-response: intermediate doses enhance anti-parasitic immunity, whereas lower or higher doses are detrimental. This widens the effective intervention window and reduces reliance on precise dosing. Together, these findings support a model in which diet-microbiome interactions can be leveraged to reduce malaria transmission and inform scalable, environmentally sustainable interventions, including L-DOPA-based sugar baits and microbiome-targeted strategies.

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UNRAVELLING THE SYMBIOTIC RELATIONSHIP BETWEEN *WOLBACHIA* AND *ANOPHELES* MOSQUITOES

Laura Charlotte Chatterley[1], Pan Prasongpolchai[1], Isabel Polly Claire Hughes[2], Seynabou Sougoufara[2], Nicola Hull[1], Janvier Bandibabone[3], Christophe Nkondjio[4], Shehu Shagari Awandu[5], Fabrizio Alberti[1], Grant Hughes[6], Eva Heinz[7], Thomas Walker[1]

[1]University of Warwick, Coventry, United Kingdom, [2]Coventry, United Kingdom, [3]Centre de Recherche en Sciences Naturelles de Lwiro, Lwiro, Democratic Republic of the Congo, [4]OCEAC, Yaoundé, Cameroon, [5]Jaramogi Oginga Odinga University of Science and Technology, Kisumu, Kenya, [6]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [7]University of Strathclyde, Glasgow, United Kingdom

Malaria, caused by *Plasmodium* parasites, is exclusively transmitted by *Anopheles* mosquitoes. Insecticide resistance and expansion of the urban vector *An. stephensi* requires novel control strategies to reduce future transmission. *Wolbachia* is a genus of endosymbiotic bacteria that inhibits pathogens in mosquitoes (including *Plasmodium* under laboratory conditions) and can induce a reproductive phenotype, cytoplasmic incompatibility (CI), which enables *Wolbachia* to spread through host populations. Recently, two novel *Wolbachia* strains were discovered in wild populations of *An. demeilloni* (*w*AnD) and *An. moucheti* (*w*AnM), this is the first evidence of genuine symbiosis between *Anopheles* and *Wolbachia*. Multiple approaches were undertaken to expand current understanding of the complex interactions between *Wolbachia* and *Anopheles* hosts. Firstly, Illumina amplicon and Oxford Nanopore Technology sequencing were utilized to show that *Wolbachia* is found in high relative abundance within the *An. demeilloni* microbiome and is present in the cephalothorax. Secondly, we provide strong evidence for CI induction in both the *w*AnD and *w*AnM strains through CI factor (*cif*) gene expression analysis in both wild *Anopheles* and in yeast cells via plasmid cloning techniques. Finally, RNASeq analysis was undertaken to assess the impact of *Wolbachia* strains on host immune gene expression in *An. demeilloni, An. moucheti* and *Drosophila simulans* infected with the closely related *w*No strain. Specifically, we investigated the expression of immune pathways shown previously to inhibit pathogen replication within mosquitoes. This work begins to build a picture of the *Anopheles*-*Wolbachia* symbiotic interactions which will ultimately enable us to assess the suitability of these novel *Wolbachia* strains for appliction in vector control methods.

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DISCLOSING THE COMPLEXITY OF METABOLIC NETWORKS AND REGULATORY MECHANISMS OF *AEDES AEGYPTI* NITROGEN METABOLISM

Patricia Y. Scaraffia[1], Jun Isoe[2], Thomas D. Horvath[3], Vlad Orlovsky[4], Paula Castillo[1], Stacy Mazzalupo[5]

[1]Tulane University, New Orleans, LA, United States, [2]The University of Arizona, Tucson, AZ, United States, [3]Baylor College of Medicine, Houston, TX, United States, [4]Helix Chromatography, Prospect Heights, IL, United States, [5]Bruker Spatial Genomics, Inc., San Jose, CA, United States

Aedes aegypti mosquitoes represent a serious public threat worldwide. They are vectors of pathogens causing several human infectious diseases. A better understanding of mosquito metabolism is needed for the successful development and implementation of metabolism-based mosquito control strategies. The application of mass spectrometry-based metabolomics and RNA interferences (RNAi) technique has allowed us to demonstrate that blood-fed *Ae. aegypti* females have evolved efficient metabolic pathways and regulatory mechanisms for coping with the high ammonia concentrations and the excess nitrogen released during a blood meal's digestion. We also discovered that deficiency of ornithine decarboxylase (ODC), the enzyme that catalyzes the rate-limiting first step for *de novo* polyamine synthesis, impairs both nitrogen metabolism and survival in *Ae. aegypti* mosquitoes. To further explore the metabolic link between polyamines and ammonia pathways, we are currently applying qPCR, western blotting, and a target quantitative LC-MS/MS method - with a new mixed-mode chromatographic separation - to examine the role of additional proteins involved in the polyamine pathway. We found that genes encoding spermidine synthase (SdS) and spermine synthase (SmS) show a distinct transcription and protein profile in mosquito tissues analyzed during the first gonotrophic cycle. We have also observed that RNAi-mediated silencing of SdS or SmS delays blood meal digestion, disrupts serine protease expression and reduces the disposal of nitrogen waste products in blood-fed females. Notably, a decreased level of SdS protein in mosquitoes by RNAi leads to a decrease in SmS protein. Conversely, a reduction of SmS protein by RNAi, decreases SdS protein levels. Moreover, RNAi-driven reduction of ODC decreases SdS and SmS protein expression, reduces expression of proteins involved in nitrogen waste disposal and decreases the concentration of specific nitrogen metabolites in mosquito tissues. Our data uncover novel crosstalk signaling regulations within the polyamine pathway and between the polyamine and uric acid pathways.

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COMPARATIVE FITNESS OF WOLBACHIA-TRANSINFECTED AND WOLBACHIA-FREE *AEDES AEGYPTI* UNDER VARIOUS LARVAL DIETS

Vidhya PT, Aravind Revi, Yazhini Gunasekaran, Amala R, Waseema Arif, Shriram AN, Manju Rahi

ICMR-Vector Control Research Centre, Puducherry, India

Wolbachia-based strategies represent a transformative and sustainable approach to dengue control, with their effectiveness demonstrated by

significant reductions in dengue incidence across multiple countries. A collaborative initiative between the Indian Council of Medical Research-Vector Control Research Centre and the World Mosquito Program (WMP) developed two *Aedes aegypti* (Puducherry) release lines carrying *w*Mel (Pud) and *w*AlbB (Pud*) Wolbachia* strains. Successful field implementation of *Wolbachia*-based vector control relies on large-scale rearing of mosquitoes with optimal fitness, where larval nutrition plays a critical role. Therefore, this study evaluated four larval diets, LD1 (fish feed), LD2 (laboratory rodent diet), LD3 (mushroom powder), and LD4 (dog biscuit + brewer's yeast) on fitness traits (wing length, body weight) and Wolbachia density in transinfected and uninfected *Aedes aegypti* across F0 and F1 generations. LD1 and LD4 significantly improved wing length in both sexes across all strains, while Wolbachia infection had no independent effect. Diet-strain interaction was largely non-significant, except for male body weight, indicating that wing length is primarily diet-driven, whereas male body weight is influenced by both diet and strain. Wolbachia density was higher under LD1 and LD2. Life table parameters including egg hatchability, pupation, adult emergence, fecundity, and survival were also significantly enhanced under LD1 and LD4. Wolbachia-infected lines (*wMel* and *wAlbB*) performed best under LD1. In contrast, uninfected females showed higher survival under LD2 (HR < 1, P < 0.001). Overall, the study results indicate that LD1 (fish feed) emerges as the optimal larval diet for mass rearing of *Wolbachia*-transinfected *Aedes aegypti*, owing to its cost-effectiveness and ready availability.

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EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) -DRIVEN HEMOCYTE AND MIDGUT CELL PROLIFERATION REVEALS A BIDIRECTIONAL CROSSTALK THAT PRESERVES BARRIER INTEGRITY IN *AEDES AEGYPTI*

George R. Samantsidis, Seijun Kim, Ben D. Chen, Nicolas S. Buchon

Department of Entomology, Cornell Institute for Host Microbe Interactions and Disease, Cornell University, Ithaca, NY, United States

The mosquito midgut must preserve epithelial integrity while responding to microbial and physiological stress, yet how regenerative and immune responses are mechanistically coordinated across compartments remains poorly understood. Here, we show that oral exposure of the flaviviral vector *Aedes aegypti* to bacterial pathogens triggers proliferative responses in the midgut and immune cell (hemocytes) compartments, aspects of which are also engaged following blood feeding. Midgut challenge increases intestinal stem cell-driven proliferation and tissue repair, while expanding midgut-associated granulocytes and proliferative hemocytes in circulation. Strikingly, this hemocyte response occurs without detectable bacterial translocation across the gut epithelium, indicating that signals arising from the challenged midgut are sufficient to stimulate systemic immune-cell proliferation. Mechanistically, these proliferative responses require Epidermal Growth Factor Receptor (EGFR) signaling, which acts in both the midgut and hemocytes to promote epithelial renewal and regulate granulocyte expansion and proliferation, respectively. Together, these observations identify a shared pathway that coordinates local tissue regeneration with systemic immune mobilization. Notably, hemocyte ablation impairs stress-induced midgut proliferative repair, demonstrating bidirectional crosstalk between the two compartments. Disruption of EGFR signaling during bacterial challenge abolishes this coordinated response and markedly reduces mosquito survival without increasing bacterial burden, indicating a defect in disease tolerance rather than resistance. Collectively, our findings identify EGFR as a key regulator of hemocyte and midgut cell proliferation and reveal a bidirectional communication between these compartments that preserves gut barrier integrity in *Ae. aegypti*. Our work establishes an experimental framework for investigating the mechanisms underlying the coordinated immune-epithelial interactions in mosquitoes.

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WILD-CAUGHT NON-ANOPHELINE MOSQUITOES CARRYING *PLASMODIUM FALCIPARUM SPOROZOITES* IN EASTERN ZIMBABWE

Sungano Mharakurwa[1], Brenda B. Makonyere[1], Trust Nyakunu[2], Tariro Chikava[1], Natasha Mbwana[1], Charmaine Chido Matimba[1], Vuyisile Mathe[1], Hieronymo Masendu[1]

[1]Africa University, Mutare, Zimbabwe, [2]Africa University, Mutare, MA, Zimbabwe

During field evaluation of a focused case-response malaria entomological surveillance approach, adult mosquitoes from several non-anopheline genera were caught, associated with villages that had the prevailing highest numbers of malaria cases for the week in sampled health centre catchments. In the current study the non-anopheline mosquito samples were subjected to analysis for malaria infection and blood meal source. It was hypothesized that, considering the adaptive potential of the malaria parasite, especially, the locally preponderant *Plasmodium falciparum*, the mosquitoes caught associated with malaria cases may be plausible candidate malaria vectors, secondar or otherwise. The focused case-response approach and standard routine entomological surveillance were conducted simultaneously every month in 24 health centre catchments of Mutasa district from 2022 - 2023, using regular prokopack aspiration and CDC light traps. Captured adult mosquitoes were identified by morphological examination with confirmatory sibling species differentiation PCR. All the mosquitoes were subjected to *P. falciparum* circumsporozoite ELISA and PCR analysis for detection of malaria sporozoites and blood meal host. A total of 781 adult *Culex* (735, 94.1%), *Mansonia* (11, 1.4%) and *Aedes* (35, 4.5%) were captured. Unlike their anopheline counterparts hardly found indoors (6.3%), the non-anophelines were caught indoors at much higher frequency (52.1%, P < 0.001, N = 4,975). One *Culex pipiens* (0.4%) was *P. falciparum* sporozoite-positive by ELISA with PCR confirmation, while a further 17 (6.3%) were positive by PCR, rivalling corresponding proportions for *Anopheles gambiae* spp (0.4% and 4.0%, respectively). Of 51 *Cx pipiens* caught blood-fed, 19 were gorged on human blood (HBI, 0.33). The findings are a first demonstration of natural sporozoite carriage in non-anopheline mosquitoes. Caught indoors and outside in areas with active malaria cases despite IRS, these culicines merit follow-up studies to ascertain whether they may play a veiled role in sustaining malaria transmission. Key words: malaria, vector, transmission, surveillance, culicine

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DO C-JUN N-TERMINAL KINASE (JNK) AND SMALL INTERFERING RNA (SIRNA) PATHWAYS ACT SYNERGISTICALLY IN MOSQUITO ANTIVIRAL IMMUNITY?

Evan Cole Y. Dumayas[1], Gladys Gutierrez-Bugallo[2], Shengzhang Dong[2], George Dimopoulos[3]

[1]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [2]Baltimore, MD, United States, [3]Johns Hopkins University Bloomberg School, Baltimore, MD, United States

Aedes aegypti mosquitoes transmit a wide variety of clinically relevant arboviruses, posing a public health threat in susceptible regions worldwide. While there is a plethora of literature on human immune defense against arbovirus infection, information on antiviral immunity within the mosquito vector remains less characterized. The siRNA pathway is considered the primary mediator of antiviral defense in mosquitoes; however, increasing evidence suggests that other immune pathways, including Toll and JAK/STAT, contribute as well. One such pathway is the c-Jun N-terminal kinase (JNK) pathway, a MAPK pathway that regulates apoptosis and has been shown to reduce viral titers in *Ae. aegypti* salivary glands. Whether the JNK pathway interacts with siRNA-mediated immunity to shape overall antiviral response in the mosquito is unclear. This research aims to elucidate how the JNK and siRNA pathways interact and contribute to antiviral immunity. We hypothesize that these pathways play distinct but convergent roles in antiviral defense, functioning either independently or synergistically. Our

approach involves comparative analyses between wild-type Liverpool mosquitoes and an Argonaute-2 knockout strain deficient in siRNA-mediated immunity. Key regulators of the JNK pathway will be silenced using morpholino oligo technology prior to dengue virus type 2 and Mayaro virus challenges via blood feeding. The phenotypes will be assessed by measuring viral titer in midguts and carcasses. Overall, this study will determine whether JNK and siRNA-mediated immunity function either independently or cooperatively in antiviral defense, providing insights that may inform the development of novel translational forms of vector control.

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FITNESS EVALUATION OF PGRP-LC'-OVEREXPRESSING TRANSGENIC *ANOPHELES GAMBIAE* FOR MALARIA CONTROL

Sida Li[1], Houhong Zhang[2], Yuemei Dong[3], Yuyang Wang[2], George Dimopoulos[4]

[1]Johns Hopkins University, Baltimore, MD, United States, [2]Baltimore, MD, United States, [3]Johns Hopkins Univ, Baltimore, MD, United States, [4]Johns Hopkins University Bloomberg School, Baltimore, MD, United States

Malaria transmission by *Anopheles gambiae* is tightly modulated by mosquito innate immunity and gut microbial interactions. The peptidoglycan recognition protein PGRP-LC is a pattern recognition receptor of the IMD pathway that controls antibacterial and anti-*Plasmodium* immune responses in the mosquito midgut. Because immune enhancement has proven effective in blocking the malaria parasites, a transgenic *Anopheles gambiae* line was generated expressing the active form of PRGP-LC in the midgut under the bloodmeal-inducible carboxypeptidase protomer, designed to activate the IMD pathway after blood feeding. Although immune enhancement is a promising malaria control strategy, sustained, ectopic, or leaky transgene expression may impose fitness costs that reduce mosquito survival and performance. We hypothesize that PGRP-LC–dependent immune enhancement may increase longevity by reducing bacterial infections, but expression at early developmental stages and non-gut tissues may also have fitness costs. To test this, we have been evaluating survival of transgenic mosquitoes across developmental stages, including larvae, pupae, and adults, as well as egg hatching, pupation, adult longevity after single and multiple blood meals, and fecundity. PGRP-LC' expression levels are being measured throughout development to assess promoter leakiness and its potential physiological consequences. The effects of PGRP-LC' overexpression on mosquito midgut microbiota are also being examined to evaluate direct or indirect impacts on *Plasmodium* through IMD pathway activation. This study defines the fitness consequences of blood meal-triggered IMD pathway activation in *An. gambiae* via PGRP-LC' overexpression and clarifies trade-offs between transgenic immune-boosting and mosquito physiology. Results will inform the design of stable and sustainable transgenic mosquitoes for malaria transmission-blocking strategies.

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EXPLORING WOLBACHIA-ANOPHELES INTERACTIONS: FUNCTIONAL VALIDATION OF WSP-INTERACTING HOST FACTORS

Mary Kefi[1], Hamidah Raduwan[2], Yu-Min Chuang[3], Han Wen[4], Caire Barreto[5], Shengzhang Dong[6], Zhiyong Xi[7], Erol Fikrig[8], George Dimopoulos[9]

[1]Johns Hopkins Bloomberg Schoolg of Public Health, Baltimore, MD, United States, [2]New Haven, CT, United States, [3]Orange, CT, United States, [4]Michigan State University, East Lansing, MI, United States, [5]Johns Hopkins University, Baltimore, MD, United States, [6]Baltimore, MD, United States, [7]Michigan State Univ, East Lansing, MI, United States, [8]Yale Sch of Medicine, New Haven, CT, United States, [9]Johns Hopkins University Bloomberg School, Baltimore, MD, United States

Wolbachia are intracellular endosymbiotic bacteria that infect a large proportion of insect species and are increasingly exploited for vector control due to their ability to interfere with pathogen transmission. While Anopheles mosquitoes were historically considered refractory to Wolbachia, recent detections in field populations and successful transinfection studies highlight the importance of understanding Anopheles-Wolbachia interactions. In particular, Wolbachia infection has been associated with reduced *Plasmodium* development, suggesting potential for malaria control strategies. To identify host factors involved in these interactions, we screened a yeast-display library of *Anopheles gambiae* secreted proteins using purified Wolbachia surface protein (WSP), a major surface protein implicated in host-symbiont interactions. This screen identified multiple putative host interactors, the majority of which are currently uncharacterized. For functional validation, the top candidate genes were subjected to RNA interference (RNAi)-mediated silencing in a *wPip*-transinfected Anopheles stephensi line. Wolbachia density was quantified using absolute quantification of WSP copy number. Of the candidates tested to date, several genes were successfully silenced, and silencing of one candidate consistently resulted in a significant reduction in *Wolbachia* levels, suggesting a role in symbiont maintenance or stability. To assess the functional relevance of this interaction for malaria transmission, *Plasmodium falciparum* infection assays were performed following gene silencing in both Wolbachia-infected and uninfected mosquitoes. Silencing of the candidate gene led to a significant increase in parasite levels, but only in the presence of *Wolbachia*, indicating that this host factor may contribute to Wolbachia-mediated parasite inhibition.Together, these findings identify a previously uncharacterized Anopheles protein that influences Wolbachia density and modulates its impact on Plasmodium infection, providing new insight into host-symbiont interactions relevant to malaria control strategies.

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DISENTANGLING ENTOMOLOGICAL AND HUMAN DRIVERS OF MALARIA TRANSMISSION ALONG THE SENEGAMBIAN BORDER: IMPLICATIONS FOR TAILORED VECTOR CONTROL

Elodie Vajda[1], Moussa Diop[2], Smita Das[3], El Hadji Amadou Niang[2], Neil Lobo[4]

[1]University of California, San Francisco (UCSF), San Francisco, CA, United States, [2]Dakar, Senegal, [3]PATH, Seattle, WA, United States, [4]Univ of Notre Dame, South Bend, IN, United States

Malaria transmission along the Senegambian border is heterogeneous (lower levels in the north, higher rates in the south). In response, Senegal and The Gambia synchronized control efforts, including mass LLIN distribution, yet the key entomological and human behavioral factors driving transmission remain underexplored. This study used the Entomological Surveillance Planning Tool (ESPT) to describe the human and entomological determinants of malaria transmission across four Senegalese sites in 2 health districts (border sites: Némataba, Ndramé Ndimb; non-border sites: Médina Dianguette, Paoskoto) towards guiding tailored malaria and vector control operations in the context of cross-border vectors and human population movements and behaviors. From July 2023 to March 2024, five cross-sectional entomological surveys were conducted using human landing catches (HLCs) integrated with human behavior observations (HBOs) on sleep patterns and LLIN usage, pyrethrum spray catches (PSCs), and insecticide resistance testing. HBOs showed that most community members were outdoors between 20h00 and midnight, with bed net use increasing markedly after this period, leaving early evening exposure largely unprotected. In Némataba and Ndramé Ndimb, the primary gap in protection was outdoors while people were awake early in the evening, followed by a secondary gap outdoors during sleep without a net. In Médina Dianguette and Paoskoto, a similar pattern of early-evening outdoor exposure was observed, with low LLIN use in Paoskoto leading to significant nighttime exposure. These results underscore the importance of maintaining high LLIN usage and, given the observed resistance to alpha-cypermethrin, support the 2025 distribution of Interceptor G2 nets. Estimated impacts of indoor residual spraying (IRS) varied widely, from 6% in Médina Dianguette to 325% in Ndramé Ndimb, indicating that the Senegal National Malaria Program should prioritize high net coverage. Additional vector control interventions to address outdoor exposure, as well as personal protection interventions for cross-border travelers, should be evaluated in pilot studies.

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LEVERAGING PUBLIC DATA FOR HABITAT SUITABILITY MODELING OF *AEDES* SPP. MOSQUITOES IN FLORIDA

Mary-Ruth Joyce, Camila Trejo Paz, Kristi Miley
University of South Florida, Tampa, FL, United States

Since 2009, there has been an increase in locally acquired cases of dengue virus in the state of Florida. In 2024, ten counties in the state documented locally acquired cases, which is the highest number of counties that have reported locally acquired cases annually. As the increase in locally acquired cases in Florida has stemmed from both the influx in travel-associated cases as well as the presence across the state of *Aedes* spp. mosquitoes capable of transmitting the virus, habitat suitability modeling of *Aedes* spp. mosquitoes is an important tool to assess areas of increased risk of local dengue transmission. MaxEnt modeling, a type of habitat suitability modeling, will be conducted using Maxent software and ArcGIS Pro. Data related to *Aedes* spp. mosquito observations will be obtained from iNaturalist, which is collected and documented by the public. Other relevant layers to be incorporated into the model include elevation, land cover data, and bioclimatic variables. The resulting habitat suitability model will be evaluated through assessing the percent contribution of each variable as well as the overall model fit. Habitat suitability modeling using mosquito observation data from iNaturalist could leverage publicly collected field data to supplement local mosquito control surveillance data when creating additional models to assess disease risk. Disease risk modeling using this type of data could also be useful during non-peak transmission time periods when mosquito abundance may be low.

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MONITORING MALARIA TRANSMISSION THROUGH A COMMUNITY-DRIVEN ENTOMOLOGICAL SURVEILLANCE IN TWO RURAL MALIAN VILLAGES

Moussa Keita[1], Aboubakr Sadik Traore[1], Amadou Sekou Traore[1], Younoussa Ziguime[1], Issa Traore[1], Yaya Zié Coulibaly[1], Gaoussou Fofana[1], Mamadou Goita[1], Mahamadou Diakite[1], Alpha Seydou Yaro[1], Adama Dao[1], Cheick Oumar Traore[2], Seydou Doumbia[2], Nafomon Sogoba[1]
[1]Infectious Disease and Medical Entomology Research and Training Center (IDMERTC), International Center for Excellence in Research, Faculty of Medicine and Odonto-Stomatology, University of Sciences, Techniques and Technologies of Bamako (USTTB), Bamako, M, Bamako, Mali, [2]University Clinical Research Center (UCRC) University of Sciences, Techniques and Technologies of Bamako (USTTB), Bamako, Mali, Bamako, Mali

Routine entomological surveillance is essential for guiding malaria vector control, but technicianled systems are costly and difficult to sustain in resourcelimited settings. This study assessed the feasibility of a communitybased approach to monitor malaria transmission in two rural Malian villages.Methods: From August to December 2025, trained community collectors conducted biweekly indoor resting mosquito collections in 40 randomly selected households per site in Koïla Bamanan (ricegrowing zone) and Dangassa (Sudanian savanna). Households were selected from a recent census using simple random sampling. Collectors received practical training on aspirator use, safe handling, and standardized labeling. During each visit, all sleeping rooms were aspirated between 06:00 and 09:00. Mosquitoes were sorted by sex and genus on site, preserved in 80% ethanol, and transported monthly to the laboratory. *Anopheles gambiae s.l.* were identified as species by PCR and tested by ELISA for *Plasmodium falciparum* sporozoites and bloodmeal origin.Results: A total of 3,042 *An. gambiae s.l*. were collected (2,931 in Koïla Bamanan; 111 in Dangassa). In Dangassa, *An. coluzzii* predominated (90.1%), followed by *An. gambiae* (5.5%) and *An. arabiensis* (4.4%). In Koïla Bamanan, all 258 PCRidentified specimens were *An. coluzzii*. The human blood index was higher in Dangassa (50.5%) than in Koïla Bamanan (26.7%) ($P < 0.0001$), whereas the entomological inoculation rate was substantially higher in Koïla Bamanan (1.380 vs. 0.011 infectious bites/person/month), indicating pronounced spatial heterogeneity in transmission. In conclusion, these findings demonstrate that communitybased vector surveillance can generate reliable entomological indicators, including species composition, feeding behavior, and EIR, and can serve as a scalable approach for guiding targeted malaria control in resourcelimited settings.

6600

COVARIATE-DRIVEN SPATIAL SAMPLING APPROACH FOR SAMPLING STATIONS PLACEMENT USING GAUSSIAN RANDOM FIELDS, POINT PROCESSES AND VORONOI TESSELLATION

Letus Laurian Muyaga[1], Luca Nelli[2], Fredros Oketch Okumu[3], Jason Matthiopoulos[4]
[1]Ifakara Health Institute, Morogoro, United Republic of Tanzania, [2]University of Glasgow, Glasgow, United Kingdom, [3]Ifakara Health Institute, Ifakara, United Republic of Tanzania, [4]university of Glasgow, GLASGOW, United Kingdom

Effective surveillance of mosquito populations is fundamental to malaria control and elimination programs as it provides the evidence base for monitoring vector densities. Spatial sampling design critically influences both the accuracy and efficiency of surveillance particularly in landscapes where environmental heterogeneity governs vector abundance. Conventional sampling including simple random, systematic grid-based, stratified, and cluster often perform poorly because mosquito populations are spatially aggregated and environmentally driven. Adaptive spatial sampling has improved surveillance by concentrating effort in areas with higher predicted vector presence thereby enhancing detection of vectors hotspots. However, it results to increased uncertainty in estimates as it fails to incorporate environmental covariates of mosquito abundance and perform poorly in low-density settings where insufficient captures make it difficult to distinguish true vector absence from sampling failure. This study presents a covariate-driven spatial sampling framework for optimizing the placement of mosquito sampling stations by using two modelling approaches; Voronoi tessellation and Gaussian random fields to represent spatially autocorrelated environmental covariates and generate sampling designs, this employs spatial point processes to produce candidate sampling locations under varying covariate-driven intensity surfaces, enabling the construction of random, regular, and clustered stations. The results demonstrate that, by explicitly accounting for spatial autocorrelation, mosquito densities and environmental drivers of vector distribution it guides station placement to maximize vector detection probability even in elimination settings where vector density is critically low, rather than distributing sampling effort uniformly across areas that may include locations without vectors an approach that yields excessive zero catches. This framework can be broadly applicable to any vector surveillance study as it improves sampling efficiency and strengthens vector surveillance systems.

6601

MALARIA VECTOR ECOLOGY AND HUMAN EXPOSURE IN A MINING AREA OF NAMPULA PROVINCE, MOZAMBIQUE

Mara Francisco Maquina[1], Pedro C.P. Aide[2], Helena Boene[3], Herminio Fernando Humberto Cossa[4], Abel Paulo Nhama[5], Monica Muataco[6], Regina Macuacua[6]
[1]Manhica Health Research Center, Maputo, Mozambique, [2]cism, Maputo, Mozambique, [3]Centro de Investigacao em Saude da Manhica, Maputo, Mozambique, [4]Manhica Health Reseach Center, Maputo, Mozambique, [5]Research Manhica Health Centre, Maputo, Mozambique, [6]kenmare, Nampula, Mozambique

In Mozambique, Nampula Province accounts for 54% of malaria cases, with mining areas like Moma and Larde districts posing particular concern due to rapid population growth and environmental changes from extractive activities. This study offers a snapshot (two months data collection) of vector ecology in a highly endemic setting, emphasizing key entomological indicators and the need for context-specific malaria control in Mozambique, while identifying gaps in community protection. Mosquito collections were carried out using four complementary methods: human-baited tent traps;

window exit traps; pyrethrum spray catches; and larval surveys of natural breeding sites. Data were complemented with direct observations of human behavior, documenting household members location (indoors vs. outdoors) and ITN usage during collection nights. *Anopheles gambiae s.l.* was the predominant (n=215), followed by *An. funestus s.l.* (n=27) and *An. tenebrosus* (n=24). Biting was mainly nocturnal peaking indoors between 18:00-22:00 and outdoors between 22:00-04:00, increasing exposure in the evening and late night. Resting behavior varied with *An. gambiae s.l.* and *An tenebrosus* rested indoors, while *An. funestus s.l.* more exophilic. High pyrethroid resistance (deltamethrin 0.05% and permethrin 0.05%) was observed. Pre-exposure to PBO restored full mortality, pointing to metabolic resistance mechanisms as the primary driver. Behavioral observations highlighted significant exposure gaps: 62% of human-vector contact occurred outdoors, where ITN protection is limited, while 38% occurred indoors. Overall, malaria transmission in the mining belt of Nampula is maintained by multiple vector species. Vector populations exhibited diverse biting and resting behaviors, with nocturnal activity outdoors where people remain unprotected. High levels of pyrethroid resistance, mediated by metabolic mechanisms, threaten the effectiveness of ITN- and IRS-based strategies. Although ITN use was reported, the high proportion of outdoor biting underscores the need for complementary interventions beyond conventional tools.

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SUSCEPTIBILITY OF *ANOPHELES GAMBIAE* SENSU LATO AND *AN. FUNESTUS* TO INSECTICIDES IN TWO ECOLOGICAL ZONES OF UGANDA WITH PARTIAL AND FULL INDOOR RESIDUAL SPRAYING (IRS) COVERAGE

Marvious Kiwanuka[1], Perpetra Akite[1], Anne Akol[2], Emmanuel Arinaitwe[3], Joaniter Nankabirwa[4], Max McClure[5], Grant Dorsey[6], Paul Joseph Krezanoski[7], Edward Thomsen[8], Catherine Maiteki-Sebuguzi[9], Moses R. Kamya[1], Neil Lobo[10]

[1]Makerere University, Kampala, Uganda, [2]Makerere University, Kampala, Uganda, Kampala, Uganda, [3]Infectious Diseases Research Collaboration, Kampala, Uganda, [4]Makerere Univ Kampala, Kampala, Uganda, [5]UCSF, San Francisco, CA, United States, [6]Univ of California San Francisco, San Francisco, CA, United States, [7]University of San Francisco, California (UCSF), San Francisco, CA, United States, [8]Malaria Elimination Initiative (MEI), Institute for Global Health Sciences, University of California, San Francisco (UCSF), Mission Hall, San Francisco, CA 94143, San Francisco, CA, United States, [9]Ministry of Health, Kampala, Uganda, [10]Univ of Notre Dame, South Bend, IN, United States

Over the past two decades, insecticide-treated nets (ITNs) and indoor residual spraying (IRS) have been the primary malaria vector control tools in Uganda. Although these interventions initially reduced transmission, their impact has plateaued or reversed in some settings, likely due to shifts in vector species, behavior, and insecticide resistance under varying ecological and intervention contexts. This study is assessing the *Anopheles gambiae* s.l. and *Anopheles funestus* susceptibility profiles and behavior in Tororo District (full IRS), Busia District (no IRS), and Amolatar District (with partial IRS coverage). Repeated cross-sectional survey are being done in which *Anopheles* larvae are being collected from multiple breeding sites in each district and reared to adults. WHO tube and bottle bioassays are performed on 3-5-day-old, non-blood-fed females using 0.05% deltamethrin, 0.05% alpha-cypermethrin, 0.1% bendiocarb, 0.25% pirimiphos-methyl, vectron 100, Sovrenta, chlorfenapyr and clothianidin; a subset was additionally tested with deltamethrin preceded by piperonyl butoxide (PBO). In addition, hourly indoor resting mosquitoes are being collected (6pm to 9 am) from six households in each district. Based on preliminary results, in these three districts, *An. gambiae* s.l. exhibited resistance to deltamethrin, while pre-exposure to PBO substantially increased mortality, indicating oxidase-mediated metabolic resistance. Despite contrasting IRS histories and ecological settings, populations from Tororo and Amolatar remained fully susceptible (100%) to clothianidin and pirimiphos-methyl, and bendiocarb mortality of 91% and 100% in Amolatar and Tororo respectively. These results demonstrate widespread pyrethroid resistance but preserved susceptibility to carbamate, organophosphate, and neonicotinoid IRS insecticides in *An. gambiae* s.l. across distinct Ugandan ecological zones. Findings support continued deployment and rotation of non-pyrethroid IRS products and the use of PBO or dual-active-ingredient ITNs.

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BACTERIAL AND FUNGAL DIVERSITY IN *ANOPHELES* LARVAL HABITATS WITH DIFFERENT PRODUCTIVITY IN MALARIA ENDEMIC SITES IN GHANA

Akua Obeng Forson[1], Yaw A. Afrane[1], Isaac K. Sraku[1], Jewelna Akorli[2], Jones Gyamfi[3], Douglas J. Perkins[4], Ivy Hurwitz[5]

[1]Department of Medical Microbiology, University of Ghana Medical School, University of Ghana, Korle-Bu, Accra, Ghana, Accra, Ghana, [2]Department of Parasitology, Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Accra, Ghana, [3]School of Health and Life Sciences, Teesside University, Middlesbrough, United Kingdom, [4]Center for Global Health, Department of Internal Medicine, University of New Mexico Health Sciences Center, Albuquerque, NM, 87106, USA., Albuquerque, NM, United States, [5]Univ of New Mexico, Albuquerque, NM, United States

Microbial communities in *Anopheles* mosquito breeding habitats play a critical role in influencing both oviposition behavior and larval development of mosquitoes. A better understanding of this relationship can be instrumental in developing innovative and sustainable mosquito control strategies. This study characterized bacterial and fungal communities across *Anopheles* larval habitats of differing productivity in southern Ghana and evaluated their association with larval abundance. Larval densities were monitored bi-weekly over 6 months, with water and larval samples collected for microbial profiling using 16S rRNA and ITS amplicon sequencing. Larval densities were monitored bi-weekly over a six-month period, during which water and larval samples were collected for microbial characterization using 16S rRNA and ITS amplicon sequencing. Distinct microbial profiles were linked to habitat productivity. Linear discriminant analysis identified *Paraclostridium bifermentans* and *Bacillus* spp. as indicator taxa of productive habitats, whereas *Acinetobacter variabilis* and *Lactococcus garvieae* were associated with non-productive sites. Among fungi, *Cladosporium halotolerans* and *Aspergillus penicillioides* were key taxa distinguishing productive habitats, while *Saccharomyces mikatae* and *Talaromyces zhenhaiensis* were characteristic of non-productive environments. Beta diversity analysis showed significant differences in bacterial community composition between productive and non-productive habitats (PERMANOVA, $p = 0.008$), whereas fungal communities showed no significant separation. These findings lay the groundwork for identifying specific bacterial and fungal taxa that could be explored for future vector control strategies in the future and underscore the potential of microbiota-informed strategies to enhance vector surveillance and targeted larval control strategies.

6604

TWENTY-FIVE YEARS (2001-2025) OF BYCATCH DATA IN GEORGIA, USA: A DESCRIPTIVE ANALYSIS OF SPATIAL AND TEMPORAL TRENDS IN *ANOPHELES* SPECIES (SPP.)

Subhika Rao[1], Daniel Impoinvil[2], Rosmarie Kelly[3], Thuy-vi Thi Nguyen[3], Sarah Zohdy[2]

[1]Centers for Disease Control and Prevention/Entomological Society of America, Atlanta, GA, United States, [2]Centers for Disease Control and Prevention, Atlanta, GA, United States, [3]Georgia Department of Public Health, Atlanta, GA, United States

Malaria, once a deadly disease for Americans, was officially eliminated from the US in 1951. Though the disease was eliminated, the vectors, *Anopheles* mosquitoes, remain. Most of the malaria cases observed in the US are travel-related cases, but there have been cases of autochthonous malaria transmission in recent years. Outbreaks occurred in Florida, Texas, Arkansas, and Maryland in 2023 and in New Jersey and Washington in 2025. Thus, surveillance of *Anopheles* within the US remains useful in managing malaria outbreaks. In this study, *Anopheles* spp. bycatch data from 2001-2025, resulting from arbovirus vector surveillance, was examined to understand the spatiotemporal trends in *Anopheles* spp. in Georgia and

assess the utility of long-term bycatch data. There were 40,095 *Anopheles* spp. trapped as bycatch from arbovirus vector surveillance activities. CDC light traps and gravid traps were consistently used throughout the study period. Light traps captured 82% of the *Anopheles* spp. collected over the 25-year study period. Based on the total number of *Anopheles* spp. and sites of collection, temporally consistent bycatch hotspots were found in the metro Atlanta and southeastern regions. *Anopheles* spp. persisted year-round, with *Anopheles crucians* consistently being the most abundant species. *An. crucians* displayed hotspot patterns distinct from *An. punctipennis* and *An. quadrimaculatus*, the next greatest species in abundance. The total numbers of mosquitoes of each of these three species peaked in 2019 or early 2020. This dataset demonstrated the value in using long-term consistently collected bycatch data. Information on the location and composition of malaria vector populations and the best trapping methods is integral in the event of a malaria outbreak, enabling targeted and effective mosquito control response. Thus, it would be beneficial to recognize bycatch data as a key component of vector control, as well as advocate for wider retention and analysis of bycatch.

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AEDES MOSQUITOES IN THE CAUVERY DELTA OF TAMIL NADU, INDIA: BREEDING DYNAMICS, INSECTICIDE RESISTANCE, AND ARBOVIRAL RISK

Rajalakshmi Anbalagan[1], Farhat S K[1], Sathya Jeevitha B[1], P K Srivastava[2], Mansi Rajendra Malik[3], Jayalakshmi Krishnan[1]

[1]Central University of Tamil Nadu, Thiruvarur, India, [2]National Vector Borne Disease Control Programme, M/o Health & FW, Govt of India, NewDelhi, India, [3]Tata Institute for Genetics and Society,Bangalore,India, Bangalore, India

Dengue remains a major public health concern across India, Including the southern state of Tamil Nadu. The Cauvery delta districts, particularly Nagapattinam and Thiruvarur, Provide favorable ecological conditions for *Aedes* (*Ae*.) mosquitoes leads to fluctuating dengue cases. Continuous entomological surveillance and monitoring of insecticide resistance are essential for effective vector control and prevention of diseases transmission. Ethical approval for the study was obtained from the Directorate of Public Health and Preventive Medicine (DPH), Chennai. *Ae.* surveillance was conducted in selected blocks of both the districts. Immature mosquitoes were collected from different breeding habitats and transported to the laboratory. The collected specimens were reared, maintained, and morphologically identified at the Vector Biology Research Laboratory (VBRL), Central University of Tamil Nadu. *Ae.* mosquitoes were tested for insecticide susceptibility using World Health Organization (WHO) standard protocols. The V1016G knockdown resistance (kdr) mutation in the voltage-gated sodium channel gene of *Ae.aegypti* using allele-specific PCR. Additionally, arboviral detection was conducted in *Ae.* mosquito pools. Discarded plastic containers were identified as the major breeding habitats. Three *Ae.* species were recorded, with *Ae. albopictus* being the predominant species in the both districts. Co-breeding of *Anophles varuna* with *Ae. aegypti* was also observed, while *Aedes vittatus* was detected in some location of Thiruvarur district. Insecticide susceptibility tests showed 100% mortality in *Ae. albopictus,* whereas *Ae. aegypti* exhibited possible resistance. Molecular analysis detected V1016G mutation in heterozygous from (VG), indicating an early stage of pyrethroid resistance development. Arbovirus positive pools were also detected in *Ae.* mosquitoes. The study highlights the predominance of *Ae. albopictus* and emerging insecticide resistance, arbovirus detection in *Ae. aegypti* populations in the Cauvery delta districts of Tamil Nadu.

6606

AUTOMATED DETECTION OF HIGH-RISK *AEDES AEGYPTI* BREEDING SITES USING DRONES

Andrew Chamberlain[1], Mehedy Hassan[2], Morgan Schillinger Tarpenning[3], Whitney Christine Weber[1], Kavita Dave Coombe[1], Muhammad Junaid[4], Giulio De Leo[5], Andang Suryana Soma[4], Ansariadi Ansariadi[4], **Joelle I. Rosser**[6]

[1]Stanford University, School of Medicine, Palo Alto, CA, United States, [2]Stanford University, Palo Alto, CA, United States, [3]Woodside, CA, United States, [4]Hasanuddin University, Makassar, Indonesia, [5]Stanford University, Monterey, CA, United States, [6]Stanford University, Portola Valley, CA, United States

Discarded tires remain a significant public health problem in tropical urban environments, serving as high-risk breeding sites for *Aedes aegypti*, the mosquitoes which transmit dengue, chikungunya, and Zika viruses. Identifying these habitats using ground-based surveillance methods can be resource-intensive and often misses fenced-off areas and tires on roofs. Using unmanned aerial vehicles (UAV) imagery collected by our research team in the Tallo sub-district of Makassar, Indonesia, an area with historically high dengue incidence, we applied deep learning algorithms to automatically detect discarded tires across the flight zone. We trained and compared U-Net++ and DeepLabV3+ convolutional neural network architectures for tire segmentation. We evaluated the models' ability to identify the locations, shapes, and spatial distribution of discarded tires in the study area. We found that both models demonstrated high prediction accuracy, with F1 scores of 0.87 and 0.82, respectively, with modestly superior performance by the U-Net++. The strong detection performance was largely attributable to the distinctive circular morphology and spectral characteristics of tires in aerial imagery, though this same characteristic occasionally led to false positives when encountering tire-mimicking objects such as outdoor air conditioning condenser units, water tank lids, and similar circular items. Identifying, locating, and eliminating potential larval breeding sites is a primary strategy for the control of *Ae. aegypti*-transmitted viruses and accurate, rapid detection of high-risk Ae. aegypti breeding sites from UAV imaging is a promising new tool to improve vector control.

6607

AFAIRY: A TARGETED OXFORD NANOPORE SEQUENCING PANEL FOR INSECTICIDE RESISTANCE GENOMIC SURVEILLANCE IN *ANOPHELES FUNESTUS*

Lilian Namuli Kayondo[1], Sanjay Curtis Nagi[1], Harun Njoroge Nganga[1], Faisal Ashraf[1], Shannan Summers[1], Emma Rogers[1], Joaniter Nankabirwa[2], Samuel Nsobya[3], Jonathan Kayondo[4], Mercy Opiyo[5], Grant Dorsey[6], Moses R. Kamya[2], Eric Lucas[1], Martin James Donnelly[1]

[1]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [2]Makerere University College of Health Sciences., Kampala, Uganda, [3]Infectious Diseases Research Collaboration, Kampala, Uganda, [4]Uganda Virus Research Institute, Entebbe, Uganda, [5]Malaria Elimination Initiative, University of California, San Francisco, San Francisco, CA, United States, [6]Univ of California San Francisco, San Francisco, CA, United States

The use of chemical vector control tools has been associated with shifts in malaria vector species composition and widespread insecticide resistance. This changing risk landscape requires an urgent response to strengthen national and regional insecticide resistance management plans to enable better tracking of the emerging and spreading threats. Genomic surveillance offers a promising avenue for studying the evolving landscape of insecticide resistance in the vectors. Previous work has shown how embedding whole-genome sequencing (WGS) within a cluster randomised trial could both track existing insecticide resistance markers and discover newly emerging resistance variants. While WGS is the most comprehensive data source, its scalability, particularly in resource-limited malaria intervention settings, is limited. Amplicon target sequencing offers a practical alternative for genomic surveillance, enabling higher throughput and simple data processing. We have developed a targeted Oxford Nanopore Technology amplicon sequencing panel (named *Afairy*), for insecticide resistance

surveillance in the major African malaria vector *An. funestus*. The panel outputs are integrated into existing bioinformatic analysis pipelines (AmpSeeker), thereby enabling rapid insecticide resistance surveillance.

6608

MALARIA ENTOMOLOGICAL SURVEILLANCE IN KINSHASA PROVINCE, DEMOCRATIC REPUBLIC OF THE CONGO: A LONGITUDINAL STUDY

Fabien Zimbombe Vulu[1], Melchior Mwandagalirwa Kashamuka[2], Kristin Banek[3], Ruthly François-Zafka[4], Pitshou Mampuya[1], Joseph Atibu[5], Michael Emch[6], Victor Mwapasa[7], Jonathan J. Juliano[8], Jonathan Parr[9], Rhoel R. Dinglasan[10], Seth Irish[11], Antoinette Tshefu Kitoto[12], Thierry Lengu Bobanga[13]

[1]University of Kinshasa, Kinshasa, Republic of the Congo, [2]Kinshasa, Republic of the Congo, [3]UNC Institute for Global Health & Infectious Diseases, Chapel Hill, NC, United States, [4]University of North Carolina at Chapel Hill, Durham, NC, United States, [5]Kinshasa Public Health School, Kinshasa, Republic of the Congo, [6]Univ of North Carolina at Chapel Hill, Chapel Hill, NC, United States, [7]Kamuzu University of Health Sciences, Blantyre, Malawi, [8]Univ of North Carolina, Chapel Hill, NC, United States, [9]University of North Carolina at Chapel Hill, Chapel Hill, NC, United States, [10]University of Florida Emerging Pathogens Institute, Gainesville, FL, United States, [11]Centers for Disease Control and Prevention, Atlanta, GA, United States, [12]Kinshasa School of Public Health, Kinshasa, Democratic Republic of the Congo, [13]University of Kinshasa, Kinshasa, Democratic Republic of the Congo

Malaria remains a major public health challenge in the Democratic Republic of Congo. To address the lack of contemporary entomological data, particularly in Kinshasa Province, this four-year study characterized key entomological parameters, including mosquito species composition, abundance, biting behavior, and insecticide resistance. We conducted a prospective observational cohort study (2018–2022) in three health areas representing rural, peri-urban, and urban settings in Kinshasa Province. Adult *Anopheles* mosquitoes were sampled using CDC miniature light traps and pyrethrum spray catches. Insecticide susceptibility of adult females was assessed using WHO tube tests, and a generalized estimating equations model evaluated the effects of year, season, site, sampling location, and collection time on mosquito abundance. A total of 1,449 *Anopheles* mosquitoes were collected: 50% in the rural health area of Bu, 49% in peri-urban Kimpoko, and 1% in urban La Voix du Peuple. The species captured were *An. gambiae* s.l. (85%), *An. paludis* (11%), and *An. funestus* (4%). *An. gambiae* s.l. was significantly more abundant indoors and after 10 PM. The overall entomological inoculation rate (EIR) was 4.6 infective bites per person per year, with *An. gambiae* s.l. contributing the highest rate. *An. paludis* showed a localized transmission peak in Impuru during the rainy season (EIR = 8.87). Resistance to DDT and pyrethroids was widespread, with an estimated 0.94 frequency of the *kdr* L1014F mutation. Bendiocarb and pirimiphos-methyl remained effective. This study confirms that *An. gambiae* s.l. remains the predominant malaria vector and plays a central role in transmission in Kinshasa. However, an emerging role of *An. paludis* in rural settings was observed. These findings underscore the need for vector control strategies tailored to diverse ecological settings. Continued entomological surveillance will be essential to track biting behavior and resistance patterns and ensure that interventions remain effective over time.

6609

DISSECTING DIFFERENCES IN *PLASMODIUM* SUSCEPTIBILITY WITHIN *ANOPHELES COLUZZII*: NON-CODING VARIATION AND THE 2LA INVERSION

Kathryn Smith Taquet[1], Renee Zakhia[2], Cameron Anderson[1], Kenneth D. Vernick[3], Christian Mitri[4], Michelle M. Riehle[1]

[1]Medical College of Wisconsin, Milwaukee, WI, United States, [2]Institut Pasteur, Paris, France, [3]Institut Pasteur, PARIS, France, [4]Paris, 75, France

The major genetic marker underlying differential susceptibility to *Plasmodium* infection in *Anopheles gambiae* and *Anopheles coluzzii* is the large monophyletic 2La inversion. The 2La inversion has been associated with multiple phenotypic traits, including vector competence, feeding and resting behavior, insecticide resistance, and thermal tolerance. However, the functional mechanisms underlying these inversion-associated phenotypes remain unclear. Using Ag1000G variant data from individual mosquitoes collected across the African continent, we found that inversion-specific variants, private to either karyotype, are enriched near the inversion breakpoints and absent in the middle of the inversion. Inversion-specific variants are distributed across exonic, intronic, and intergenic regions. However, none of the exonic variants appear to have a significant predicted effect on protein function, directing attention to non-coding variation. A candidate regulatory region located at the distal end of the inversion and harboring three inversion-specific SNPs was selected for functional characterization. Of these three inversion-specific SNPs, a single SNP is necessary and sufficient for a significant difference in enhancer activity detected between 2La and 2L+ alleles. This single SNP occurs at a conserved position within a computationally predicted transcription factor binding site. EMSAs using probes that differ by a single nucleotide display distinct shift patterns, with only the probe corresponding to the 2La sequence capturing a protein-DNA complex. Experimental modulation of enhancer activity identified target genes and revealed influence on Plasmodium susceptibility.

6610

USING IDENTITY-BY-DESCENT TO ASSESS POPULATION STRUCTURE IN THE MALARIA VECTOR *ANOPHELES GAMBIAE*

Mohammed Ndiaye[1], Bing Guo[2], Jean-Paul Courneya[1], Robert McCann[3]

[1]Center for Vaccine Development & Global Health, Baltimore, MD, United States, [2]Baltimore, MD, United States, [3]University of Maryland SOM, Baltimore, MD, United States

Malaria remains a major public health burden in Africa, where vector control strategies are increasingly threatened by widespread insecticide resistance. Understanding population structure in African malaria vectors is imperative for informing sustainable vector control strategies. However, current population genetic approaches lack the resolution required to capture fine-scale population structure between vector populations. Identity-by-descent (IBD) methods offer a framework for resolving fine-scale population structure. While IBD methods have been widely applied in human genomics, their use for studying mosquito populations has been limited. In this study, we assessed fine-scale population structure in *Anopheles gambiae*, one of the primary malaria vectors in Africa. We analyzed whole-genome sequence data from MalariaGEN, a publicly available data set, including mosquito populations sampled across multiple African countries. Prior to IBD analysis, we performed principal component analysis (PCA) based on single nucleotide polymorphisms (SNPs) to visualize population structure and assess clustering patterns. We applied hmmibd-rs to phased haplotype data and evaluated the sensitivity of the output to changes in key biological parameters to infer identity-by-descent. The PCA revealed clustering of samples consistent with geographic location. The IBD corroborated this pattern, with higher levels of shared ancestry observed within districts than between them. These results suggest that *Anopheles gambiae* population structure is shaped by spatial distance among sample locations, consistent with a pattern of isolation-by-distance.

6611

EVIDENCE OF DENGUE VIRUS TRANSMISSION AND A DIVERSE *AEDES* MOSQUITO VIROME ON THE DEMOCRATIC REPUBLIC OF CONGO-ANGOLA BORDER

Wenqiao He[1], Thierry Lengu Bobanga[2], Anne Piantadosi[3], Zachary R. Popkin-Hall[1], Fabien Z. Vulu[4], Matthew H. Collins[5], Melchior M. Kashamuka[6], Antoinette Tshefu Kitoto[7], Jonathan J. Juliano[8], Jonathan Parr[1]

[1]University of North Carolina at Chapel Hill, Chapel Hill, NC, United States, [2]University of Kinshasa, Kinshasa, United States, [3]Emory University, Atlanta, GA, United States, [4]University of Kinshasa, Kinshasa, Democratic Republic

of the Congo, [5]Emory University School of Medicine, Atlanta, GA, United States, [6]Kinshasa School of Public Health, Kinshasa, Democratic Republic of the Congo, [7]Kinshasa School of Public Health, Kinshasa, United States, [8]Univ of North Carolina, Chapel Hill, NC, United States

Aedes mosquitoes are widely distributed across the Democratic Republic of Congo (DRC), and are major vectors of dengue (DENV), Zika, chikungunya (CHIKV), and yellow fever (YFV) viruses. While the high burden of malaria in the DRC receives considerable attention, arboviruses remain understudied. In the setting of recent CHIKV and YFV outbreaks in southwestern DRC, we collected *Aedes* mosquitoes in three areas of Kimpese, DRC, near the Angola border, to investigate their virome. From 37 total mosquito pools, 8 pools with a total of 155 mosquitoes were randomly selected from three collection sites for metagenomic RNA sequencing. RNA was extracted from field pools, as well as an uninfected laboratory-reared *A. aegypti* mosquito pool and a water sample as controls. *Aedes* rRNA was depleted using a probe-based hybridization assay, and sample libraries prepped before Illumina NovaSeq sequencing. Data was analyzed using an in-house pipeline employing trimming; host, human and contaminating contig removal; and taxonomic identification using the KrakenUniq standard NCBI-nt database. High-confidence DENV reads and human blood meals were found in six (75%) and eight (100%) pools, respectively. Consensus sequences generated from the BLAST-confirmed reads mapping to DENV-2 from one mosquito pool (K5) closely matched a Malaysian sequence, while short DENV-4 reads (<100 bp) detected in all six pools showed high similarity to the 5′ UTR of a Thailand strain. Real-time PCR targeting the DENV polyprotein gene, distinct from genomic regions included in the detected DENV reads, confirms DENV RNA in the three mosquito pools with highest DENV read counts in metagenomic analysis. We find diverse mosquito viromes including other known and putative human and animal viruses, such as bat faecal associated dicistrovirus 4. Our findings provide strong evidence of endemic DENV transmission along the DRC-Angola border and illustrate the potential of wild-caught mosquitoes for xenosurveillance of emerging pathogens.

6612

A RECIPROCAL H3K27 METHYL-ACETYL SWITCH ORCHESTRATES THE DIAPAUSE LANDSCAPE IN THE FAT BODY OF *CULEX PIPIENS*

Moumita Sharmin Jhara[1], Prabin Dhungana[1], Gavin Geddes[2], Kaylee Rae McKay[2], Cheolho Sim[1]

[1]Baylor University, Waco, TX, United States, [2]Waco, TX, United States

West Nile Virus (WNV) remains the most widespread mosquito-borne disease in the United States, and its seasonal persistence is maintained, in part, by the overwintering infected females of *Culex pipiens*. Despite extensive knowledge of the hormonal reprogramming that initiate diapause, how these signals are translated into durable transcriptional states is still unresolved. Epigenetic regulators that regulate chromatin environments and thereby enable the genome to maintain transcriptionally active or repressed configurations have been reported as one of the key factors in diapause biology. Our previous work has demonstrated the reduced abundance of repressive H3K27 methylation (H3K27me2/3) marks in the fat body of diapausing mosquitoes. These diminished methylation levels, in notion, could be accompanied by the gain of an activation-associated modification at the same residue. To test this hypothesis, we compared histone acetylation levels in ovary and fat body tissues from diapausing and non-diapausing females by Western blot analysis. H3K27ac was markedly elevated in the fat body of diapausing females relative to non-diapausing counterparts, whereas ovarian tissues did not show a comparable pattern. This enrichment occurred in parallel with the previously observed reduction in H3K27 methylation, highlighting a reciprocal relationship between repressive and activating modifications at H3K27 in the fat body during diapause. These findings suggest that diapause-associated physiology is accompanied by coordinated remodeling of H3K27-linked chromatin states, rather than by isolated changes in a single histone mark and provides a dynamic environment at chromatin level. Future studies integrating locus-specific chromatin profiling and functional perturbation will be required to determine how these modifications regulate diapause-associated gene expression programs. Defining this regulatory axis may provide new insight into how seasonal physiological states are stabilized in mosquito vectors and inform strategies aimed at disrupting overwintering persistence and early-season WNV transmission.

6613

MULTIOMICS DISSECTION OF EXTREME PYRETHROID RESISTANCE AND FITNESS IN *ANOPHELES FUNESTUS*

Vanessa Brigitte Ngannang-Fezeu[1], Hervé Tazokong[1], Blanche Ndifo[2], Gadji Mahamat[1], Theofelix Tekoh[1], Sonia Ngongang-Yipmo[1], Murielle Wondji[1], Leon Mugenzi Jean[3], Magellan Tchouakui[4], Charles S. Wondji[5]

[1]Centre for Research in Infectious Diseases (CRID), Yaounde, Cameroon, [2]German Center for Infection Research, Tübingen, Germany, [3]Center for Research in Infectious Diseases CRID, Yaounde, Cameroon, [4]Centre for Research in Infectious Diseases (CRID), Yaoundé, Cameroon, [5]Liverpool Sch of Tropical Medicine, Liverpool, United Kingdom

Widespread pyrethroid resistance in major malaria vectors threatens the effectiveness of current vector control tools. To elucidate mechanisms driving extreme resistance escalation, we established a superresistant *Anopheles funestus* colony (Delta10X_Fz_Selected) by selection with deltamethrin 0.5% (10X) and applied an integrated multiomics approach. Insecticide bioassays showed that Delta10X_Fz_Selected was markedly more resistant than the Fz_Unselected colony to pyrethroids, carbamates, and, to a lesser extent, neonicotinoids, while both colonies remained fully susceptible to organophosphates, organochlorines, and pyrroles. Cone bioassays revealed substantially reduced efficacy of insecticide-treated nets against Delta10X_Fz_Selected compared with Fz_Unselected. Longevity assays indicated a fitness advantage, with Delta10X_Fz_Selected adults surviving longer (median 39 days) than Fz_Unselected (26 days), suggesting that extreme resistance does not incur a detectable survival cost under laboratory conditions. Transcriptomic analysis of Delta10X_Fz_Selected identified strong overexpression of multiple detoxification gene families, including cytochrome P450s (notably *CYP6P9a/b* and *CYP6Z4*), glutathioneStransferases, carboxylesterases, and sulfotransferases, alongside the emergence of additional, previously unimplicated gene families associated with resistance escalation. Wholegenome sequencing revealed clear signatures of a selective sweep in Delta10X_Fz_Selected relative to Fz_Unselected, and RTqPCR plus polymorphism analysis confirmed markedly elevated expression and reduced genetic diversity at *CYP6P9a* and *CYP6P9b*, with *CYP6P9b* showing >200fold overexpression. These convergent lines of evidence implicate intensified metabolic detoxification, driven by both known and novel loci, as the primary mechanism underpinning extreme resistance and its associated fitness gain. Our findings have direct implications for the design and deployment of nextgeneration malaria vector control tools capable of overcoming escalating resistance in *An. funestus*.

6614

BEYOND THE BED NET: EXPANDING THE ENTOMOLOGICAL LENS ON *ANOPHELES COUSTANI* IN OUTDOOR MALARIA TRANSMISSION

Renee LMN Ali[1], Margaret V. Becker[1], Shelby Morgan Cagle[1], Mary E. Gerbhardt[1], Hunter Chilusu[2], James S. Lupiya[2], Mbanga Muleba[2], Westone Phanuel Hamwata[2], William J. Moss[1], Douglas E. Norris[1]

[1]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [2]National Health Research and Training Institute, Ndola, Zambia

As malaria programs transition from control to elimination, understanding transmission beyond the protection conferred by bed nets is critical to sustaining gains and preventing resurgence. There is growing evidence that malaria transmission persists, at least in part, due to outdoor biting and adaptive shifts in vector behavior outside the protective reach of indoor-focused interventions such as long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS). More specifically, exophagic species such as

Anopheles coustani are increasingly recognized as contributors to residual malaria transmission in regions of southern and eastern Africa. However, the distribution and population genetic structure of this species across Africa remains poorly understood, limiting our ability to assess the role this species plays in regional transmission. Adult *An. coustani* specimens were collected from several geographically distinct regions of central and southern Africa. More than 15 new mitochondrial genome sequences for *An. coustani* were generated and analyzed. The cytochrome oxidase I (COI) gene was further targeted for haplotype-based analyses with additional COI sequences available from Genbank across the known geographic distribution of this African anopheline. Analyses revealed the presence of 31 COI haplotypes, with 4 shared haplotypes across distant regions and several peripheral haplotypes forming distinct clusters that do not fully correlate with geographic origin. Additional data are needed to fill in the distribution gaps to better assess connectivity across the populations sampled and the role of ecology and behavioral plasticity in driving genetic divergence. This study provides the framework for such an assessment.

6615

INVESTIGATING AND VALIDATING THE ROLES OF GENES THAT ARE HIGHLY REGULATED UPON *PLAMODIUM FALCIPARUM* INFECTION USING RNAI-MEDIATED GENE SILENCING

Yuyang Wang[1], Yuemei Dong[2], Madhavinadha Prasad Kona[1], George Dimopoulos[3]

[1]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [2]Johns Hopkins Univ, Baltimore, MD, United States, [3]Johns Hopkins University Bloomberg School, Baltimore, MD, United States

Transcriptomic and functional genomic analysis identifies mosquito genes modulating *Plasmodium falciparum* developmentYuyang Wang, Yuemei Dong, Madhavinadha Prasad Kona, George DimopoulosMalaria remains a global health challenge, with transmission of malaria parasites to humans primarily mediated by Anopheles mosquitoes infected with *Plasmodium falciparum*. Understanding the molecular interactions between the parasite and its mosquito vector is critical for developing innovative control strategies. We performed RNA-seq-based whole-transcriptome profiling of *Anopheles gambiae* midguts and carcasses 24 h post-infection with *P. falciparum* (human malaria parasite) or *P. berghei* (rodent malaria). Comparative R-based analysis revealed mosquito transcripts with both conserved and species-specific responses. Upregulated genes in the mosquito midgut displayed broadly similar functional profiles for *P. falciparum* and *P. berghei*, indicating shared early midgut epithelial responses to invasion by both parasites. Among them, 18 genes — either strongly regulated specifically by *P. falciparum* or commonly by both parasite species — were selected for functional validation. Functional genomics studies were performed by RNAi-mediated gene silencing: gene-specific primers were designed for dsRNA synthesis, the resulting dsRNAs were injected into 3-d-old adult *A. gambiae*, followed by *P. falciparum* infection using standard membrane feeding assays and oocyst quantification at 8 days post-infection. Results showed that silencing four genes, including the gene *ACYL,Tsf1,and ABCC*, significantly reduced *P. falciparum* oocyst load, whereas silencing two genes, *HPX8* and *STER*, significantly increased oocyst numbers, consistent with host-restrictive and host-facilitative roles, respectively. These results provide functional evidence that infection-responsive mosquito genes modulate parasite development in the vector. Furthermore, they highlight novel candidate genes that may serve as targets for transmission-blocking interventions, offering promising opportunities for innovative vector control strategies.

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MOLECULAR CHARACTERIZATION OF MALARIA VECTORS IN MAINLAND TANZANIA: IDENTIFICATION OF *ANOPHELES* SIBLING SPECIES, DETECTION OF *PLAMODIUM FALCIPARUM*, AND GENOMIC SCREENING OF PARASITES IN INFECTED MOSQUITOES

Baraka W. Mbuba[1], Happyness A. Mwailunga[1], Rule Budodo[1], Catherine Bakari[1], Hussein J. Semboja[1], Laurencia S. Tangawizi[1], Gideoni I. Maulidi[1], Ninja S. Bisaga[2], Dativa Pereus[3], Angelina J. Kisambale[1], Salehe S. Mandai[1], Gervas Chacha[2], Doris David Mbata[2], Daniel P. Challe[4], Daniel A. Petro[5], Solomon Mwakasungula[1], Bernard Batengana[4], Yahya A. Derua[4], Rashid A. Madebe[2], Daniel Mbwambo[6], Sijenunu Aaron[6], Abdallah Lusasi[6], Samwel Lazaro[6], Stella Kajange[6], Celine I. Mandara[2], Misago D. Seth[2], Deus S. Ishengoma[1]

[1]Ifakara Health Institute, Dar es Salaam, United Republic of Tanzania, [2]National Institute for Medical Research, Dar es Salaam, United Republic of Tanzania, [3]Muhimbili University of Health and Allied Sciences, Dar Es Salaam, United Republic of Tanzania, [4]National Institute for Medical Research, Tanga, United Republic of Tanzania, [5]University of Dar es Salaam, Dar es Salaam, United Republic of Tanzania, [6]National Malaria Control Programme, Dodoma, United Republic of Tanzania

Rising insecticide resistance and evolving vector dynamics necessitate innovative monitoring of vector infectivity and genetic diversity to guide malaria elimination. This study established malaria molecular surveillance (MMS) capacity and generated baseline entomological data through cross-sectional surveys across five regions of Tanzania with varying transmission intensities. Mosquitoes were collected in 2023 (August - October), and 2025 (June - August) through indoor trapping using CDC light traps and outdoors with Furvela tent traps. In 2023, 9352 malaria vectors were collected, and 1860 of these were randomly selected for molecular analysis, with 404 (21.7%) *Anopheles gambiae* complex and 1314 (70.6%) *An. funestus* group. For detection of sibling species in *An. gambiae* complex, majority 75% were *An. arabiensis* and the rest were *An. gambiae* s.s. 25%. *An. funestus s.s.* was the dominant species (96.5%) in the *An. funestus* group, and the rest were *An. parensis* 0.08%, *An. rivulorum 1%* and *An. leesoni* 0.7%. In 2025, 6201 mosquitoes were collected and 92.4% were *An.funestus* group, and 2087 randomly selected mosquitoes were used for sibling species detection (including 469 *An.gambiae* complex and 1618 *An. funestus* group). For the *An.gambiae* complex, *An. arabiensis* were 61.4%, and the rest were *An. gambiae s.s. 38.4%*. In the *An.funestus* group, 88.3% were *An.funestus s.s.*, *An. parensis* 7.3%, *An. rivulorum 3.4%* and *An. leesoni* 0.06%. Detection of *Plasmodium falciparum* infection using qPCR was done for 1799 samples collected In 2023, while 2087 were analysed in 2025. Of these, 9 (0.5%) and 15 (0.72%) samples were positive in 2023 and 2025, respectively. The annual entomological inoculation rate (EIR) was higher in Kyerwa - Kagera, reaching 244.76 infectious bites per person per year in 2023 and 31.52 in 2025. In 2025, positive samples (15) were sequenced using Molecular Inversion Probes, and no known validated or candidate artemisinin resistance mutations were detected. The findings show a clear predominance of *An. funestus s.s.* and sustained EIR in Kagera emphasizing the need for strengthened, targeted vector control and MMS in Tanzania.

6617

GENERATION AND CHARACTERIZATION OF *CRYPTOCHROME 1* KNOCK-OUT MUTANT *ANOPHELES GAMBIAE*

Cassandra Kaplan[1], Shengzhang Dong[2], Mary Kefi[2], George Dimopoulos[3]

[1]Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, United States, [2]Baltimore, MD, United States, [3]Johns Hopkins Univ Sch of Public Health, Baltimore, MD, United States

Anopheles gambiae is a major malaria vector in sub-Saharan Africa. Although insecticide-based vector control has been central to malaria prevention, increasing insecticide resistance in mosquito populations

highlights the need for new control strategies. Genetic modification of mosquitoes to reduce vector competence offers a promising alternative approach to limiting malaria transmission. Proteomic analyses comparing the mosquito midgut after an infected blood meal versus a naïve blood meal identified *Cryptochrome 1* (*Cry1*), a key regulator of circadian rhythm. Previous studies showed that reduced *Cry1* expression by RNA interference increased *Plasmodium falciparum* infection burden in *An. gambiae*, suggesting that *Cry1* may influence parasite susceptibility. Here, we generated *Cry1* mutants using a CRISPR-Cas9 strategy by crossing a guide RNA-expressing (U6-gRNA) line with a germline Cas9 (vasa-Cas9) line. Our results demonstrate successful mutation of *Cry1* in the offspring of the crossed parental lines. We are currently establishing a stable *Cry1* mutant line and will evaluate its susceptibility to *P. falciparum* infection as well as fitness traits relevant to mosquito development, fecundity and survival. These studies will provide insight into the role of *Cry1* in mosquito biology and its potential contribution to malaria parasite infection and transmission.

6618

SEASONAL PHOTOPERIOD DIFFERENTIALLY MODULATES CORE CIRCADIAN CLOCK DYNAMICS AND PARAMETERS OF BEHAVIORAL RHYTHMICITY IN *CULEX PIPIENS* AND *CX. QUINQUE FASCIATUS* MOSQUITOES

Gavin Geddes[1], Prabin Dhungana[1], Moumita Sharmin Jhara[2], Kaylee Rae McKay[2], Cheolho Sim[3]

[1]Baylor University, Waco, TX, United States, [2]Waco, TX, United States, [3]Baylor Univ, Waco, TX, United States

Within temperate regions, insect populations must employ complex physiological and behavioral mechanisms to anticipate and survive harsh winter conditions. A central mechanistic question in understanding this process is how populations accurately measure and respond to seasonal changes in daylength. The endogenous circadian clock is proposed to function as the primary photoperiodic timer, measuring changes in daylength to align physiology and behavior with the appropriate season. Although work in *Drosophila melanogaster* has provided mechanistic insights implicating the circadian clock in regulating daily behavior and neurosecretory cell activity, its role in regulating seasonal dormancy remains poorly resolved in organisms exhibiting robust photoperiodism. The mosquito *Culex pipiens,* a vector of West Nile Virus, undergoes adult reproductive diapause in response to shortening daylengths of early fall, a state characterized by arrested ovarian development, altered behavior, enhanced stress tolerance, lipid accumulation, and extended lifespan. However, the closely related *Cx. quinquefasciatus* maintains reproductive physiology year-round and lacks capacity to enter diapause. To identify mechanisms by which the circadian clock regulates photoperiodic diapause, we compared behavioral rhythmicity parameters and core clock gene expression dynamics between both species under diapause-inducing and diapause-averting photoperiods. We find that seasonal photoperiod differentially modulates diel activity patterns and circadian gene oscillation between species, with *Cx. pipiens* exhibiting greater photoperiod-dependent shifts in rhythmicity and transcriptional cycling than *Cx. quinquefasciatus*. These results demonstrate that photoperiodic regulation of circadian dynamics is associated with diapause capacity and suggest that evolutionary divergence in circadian entrainment and output pathways contributes to species-specific seasonal responses. This work provides insight into how circadian networks encode seasonal time and coordinate seasonal behavior in disease-vectoring mosquitoes.

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CHARACTERIZING *WOLBACHIA* SURFACE PROTEIN-INTERACTING *AEDES AEGYPTI* PROTEINS THAT REGULATE *WOLBACHIA* DENSITY AND DENGUE VIRUS INFECTION

Bijun Ling[1], Shengzhang Dong[2], Hamidah Raduwan[3], Cindy Megumi Kimura[4], Yu-Min Chuang[5], Erol Fikrig[6], George Dimopoulos[7]

[1]Johns Hopkins University, Baltimore, MD, United States, [2]Baltimore, MD, United States, [3]New Haven, CT, United States, [4]Cindy, Botucatu, SP, Brazil, [5]Orange, CT, United States, [67]Johns Hopkins Univ Sch of Public Health, Baltimore, MD, United States

Aedes aegypti is the primary vector for dengue virus (DENV), a global health threat for which specific therapeutics and widely effective vaccines remain limited. *Wolbachia*, a maternally inherited intracellular bacterium present in more than 60% of insect species, has emerged as a promising biocontrol strategy for reducing DENV and other arbovirus transmission by *Ae. aegypti*. This approach acts primarily through cytoplasmic incompatibility and pathogen interference. However, the long-term success of *Wolbachia*-based interventions may be challenged by climate change, particularly elevated temperatures that can reduce *Wolbachia* stability and maternal transmission, making strain selection and adaptive deployment increasingly important. To identify mosquito host factors involved in *Wolbachia* maintenance and antiviral activity, we screened *Wolbachia* surface protein (WSP) against a genetically barcoded yeast surface display library comprising 3,452 *Ae. aegypti* exoproteins, including extracellular and secreted proteins, and identified dozens of candidate WSP-interacting mosquito proteins. In this study, we characterize the roles of selected candidate genes in regulating *Wolbachia* density and DENV infection using RNA interference-mediated gene silencing. These findings will provide molecular insight into *Wolbachia*-mosquito interactions and improve our understanding of how host factors influence *Wolbachia* persistence and its ability to suppress arbovirus transmission.

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SMALL RNA NETWORKS LINK INSULIN SIGNALING TO METABOLIC SHIFTS IN DIAPAUSING *CULEX PIPIENS*

Kaylee Rae McKay[1], Moumita Sharmin Jhara[2], Gavin Geddes[2], Cheolho Sim[1]

[1]Baylor University, Waco, TX, United States, [2]Waco, TX, United States

To survive harsh winter conditions, the mosquito Culex pipiens enters a state of adult reproductive diapause, triggered by environmental cues such as shortening photoperiods and declining temperatures. This dormancy is characterized by arrested ovarian development and extensive physiological remodeling including shifts in metabolism, stress tolerance, and immune function. Nonetheless, the regulatory mechanisms coordinating these diapause-associated changes, particularly the small non-coding RNA networks that link gene expression across tissues, remain poorly defined. To address this, we use small RNA sequencing datasets that we previously generated from the fat bodies and ovaries of diapausing and non-diapausing female Cx. pipiens to define how small non-coding RNAs are organized into regulatory networks associated with diapause. Our approach centers on the miRNA cqu-miR-8-5p as a key reference point and examines how other small RNAs and predicted gene targets are organized around it. Comparative analyses reveal that small RNAs and their predicted gene targets are organized around cqu-miR-8-5p into coordinated regulatory networks associated with energy homeostasis, reproductive suppression, and stress responses. Consistent with the established role of miR-8 in insulin signaling, these networks link small RNA regulation to metabolic pathways central to diapause biology, establishing a cqu-miR-8-5p-centered regulatory framework that underlies diapause in Cx. pipiens.

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GENOMIC SIGNATURES OF RESISTANCE: SPECIES-SPECIFIC ADAPTATION IN *ANOPHELES FUNESTUS* AND *AN. ARABIENSIS* ACROSS KENYA

David P. Tchouassi[1], Mahamat Gadji[2], WONDJI Charles Sinclair[3]

[1]International Centre of Insect Physiology & Ecology, Nairobi, Kenya, [2]Centre for Research in Infectious Diseases, Yaounde, Cameroon, [3]Yaounde, Cameroon

Malaria surveillance remains a key pillar of global malaria technical strategy (GTS), that includes tracking evolution of insecticide resistance in primary vectors. Here, we performed pool sequencing on *Anopheles funestus* and *An. arabiensis* two major malaria vectors in East Africa. The samples were collected across major epidemiologic zones in the country spanning geographic areas within and on either side of the Great Rift Valley, a known geographic barrier to gene flow in vector populations. Population structure was assessed using principal component analysis. While *An. arabiensis* exhibited geographic structuring along the Rift Valley, in contrast to *An. funestus* that we uncover geographically proximal but genetically distinct populations. Selection in known resistance loci showed weak variation in *An. funestus* in contrast to *An. arabiensis* with detection of a novel signal. Our study demonstrates species-specific genomic architectures underlying insecticide resistance. *An. funestus* exhibits historical, polygenic selection patterns, whereas *An. arabiensis* shows ongoing, region-specific adaptation at both known and novel loci. These findings underscore the need for tailored, species and region-specific vector control strategies, continuous molecular surveillance, and incorporation of local genomic data into resistance management frameworks to sustain malaria control efforts in Kenya.

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GENOMIC EVIDENCE OF AFRICAN AND GLOBAL ANCESTRY IN *AEDES AEGYPTI* POPULATIONS FROM THE HORN OF AFRICA

Babatunde Lawal Oriyomi[1], Said Ali[2], Solomon Yared[3], Ayomikun Emmanuel Aderounmu[1], Tamar E. Carter[1]

[1]Baylor University, Waco, TX, United States, [2]Ministry of Health Development, Hargeisa, Somalia, [3]Jigjiga University, Jigjiga, Ethiopia

Patterns of connectivity and ancestry in *Aedes aegypti* remain poorly resolved across the Horn of Africa, a region experiencing increasing arboviral transmission. To address this gap, we analyzed whole-genome sequence data from populations in Ethiopia (Semera, Jigjiga, and Erer) and Somaliland (Burao, Hargeisa, and Berbera) alongside globally distributed populations to investigate regional population structure, genetic differentiation and patterns of shared ancestry. Principal Component Analysis (PCA) and admixture analyses revealed clear regional population structure. Ethiopian and Somaliland populations forming closely related clusters within the broader African lineage. Admixture results further indicated that Horn of Africa populations share ancestry components with Angola, suggesting a shared African lineage. Phylogenetic analysis supported these findings, showing that Ethiopian and Somaliland populations cluster within a closely related Horn of Africa clade. Genome-wide nucleotide diversity analyses revealed variation in genetic diversity among regional populations, with Ethiopian populations exhibiting the highest diversity and Burao showing intermediate levels. Pairwise fixation index (F_{st}) estimates indicated low genetic differentiation between Ethiopian and Somaliland populations but substantial differentiation between Horn of Africa populations and global populations. These results provide new insights into the population genetic structure of *Ae. aegypti* in the Horn of Africa and establish an important genomic baseline for understanding the evolutionary dynamics of this vector in East Africa.

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ASSESSING TOXIN-EXPRESSING TRANSGENIC MOSQUITOES AS CANDIDATES FOR MALARIA CONTROL

Emelia Pearson[1], Houhong Zhang[2], Yuemei Dong[3], George Dimopoulos[4]

[1]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [2]Baltimore, MD, United States, [3]Johns Hopkins Univ, Baltimore, MD, United States, [4]Johns Hopkins University Bloomberg School, Baltimore, MD, United States

Malaria remains a global health challenge causing substantial morbidity and mortality, especially in sub-Saharan Africa. New control strategies are needed to overcome current limitations, including *Plasmodium* drug resistance and the incomplete efficacy of existing transmission-blocking approaches. The overarching focus of my research is malaria vector control through the genetic engineering of *Anopheles* mosquitoes as tools for reducing parasite transmission. Previous published studies have shown genetically modified *Anopheles* mosquitoes expressing anti-*Plasmodium* effector genes can reduce *Plasmodium falciparum* transmission. However, having additional transgenic mosquito-based concepts available in the malaria control toolbox is necessary. One potentially promising approach is to engineer mosquitoes that die following *P. falciparum* infection, with the design involving toxin expression in a *Plasmodium* infection-inducible manner. To identify the best toxins for this approach, several transgenic lines were generated as a proof of principle, expressing insect-specific toxins in the mosquito after a blood meal. This pilot study aims to identify the best toxin candidates and determine whether toxin expression imposes unintended biological burdens. Four transgenic lines were generated previously, expressing toxins under the blood meal inducible promoters: the carboxypeptidase (Cp) promoter for midgut expression and Vitellogenin (Vg) promoter for fat-body expression. In this study, mosquito survival after single and multiple blood meals is measured to evaluate toxin efficacy. In addition, the effects of toxin expression on *P. falciparum* infection are being determined. Furthermore, the fitness costs across life stages, including development and fecundity, are also being assessed. Future work will focus on developing the two best-performing toxins under dual *Plasmodium*-inducible promoter systems and assessing their utility for vector control.

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INTERROGATION OF MOSQUITOCIDAL FACTORS FROM CHROMOBACTERIUM CSP_P

Chenxi Sun[1], Vandana Prasad[2], Madhavinadha Prasad Kona[2], George Dimopoulos[3]

[1]Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD, United States, [2]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [3]Johns Hopkins Univ Sch of Public Health, Baltimore, MD, United States

Chemical insecticide resistance poses an increasing hurdle for controlling mosquitoes and the diseases they transmit and also causes non-target effects and environmental impacts. Chromobacterium sp. Panama (Csp_P) has emerged as a promising natural microbial biopesticide for controlling mosquitoes and other pest insects. A non-live crude preparation of Csp_P kills both larval and adult stages of Anopheles, Aedes, and Culex mosquito vectors through heat-stable factor(s). However, the molecular nature of these insecticidal factors remains unknown.We employed comprehensive genomic, genetic, and biochemical approaches to identify candidate Csp_P-produced factors responsible for mosquito killing:Comparative genomics between Csp_P and non-mosquito-killing Chromobacteria identified candidate biosynthetic gene clusters potentially responsible for insecticidal activity.Comparative transcriptomics under Csp_P culturing conditions that either produce or do not produce insecticidal activity revealed candidate genes involved in mosquito-killing metabolite production.Mutagenesis of certain Csp_P biosynthetic gene clusters abolished mosquitocidal activity, confirming their functional relevance. Bioassay-guided fractionation, coupled with mass spectrometry (MS) and nuclear magnetic resonance (NMR), identified candidate metabolites

potentially responsible for the mosquitocidal effect.Ongoing studies include assessing the activity and specificity of synthesized candidate molecules, determining whether their action is independent or synergistic, and investigating the mode of action of these novel factors.

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INTROGRESSION OF ADAPTIVE ALLELES IN THE *CULEX PIPIENS-QUINQUEFASCIATUS* HYBRID ZONE

Benjamin Gregory[1], Megan Fritz[2]
[1]University of Maryland, College Park, MD, United States, [2]Laurel, MD, United States

Climate-driven shifts in hybrid zones can facilitate the spread of adaptive alleles in disease vectors, with implications for transmission risk. In the eastern United States (U.S.), the hybrid zone between *Culex pipiens* and *Culex quinquefasciatus*—primary vectors of West Nile virus—spans the mid-latitudes. However, habitat suitability for the southern-adapted *Cx. quinquefasciatus* may also vary locally along urbanization gradients due to urban heat island effects. To test for adaptive introgression between species, we conducted pooled whole-genome sequencing of *Cx. pipiens* assemblage populations from 24 sites across the Baltimore-Washington metropolitan region (BW), and compared them to reference populations of *Cx. pipiens* and *Cx. quinquefasciatus* from the latitudinal extremes of their U.S. ranges. Genome-wide scans identified a dense cluster of loci strongly associated with maximum summer temperature. Analysis of this genomic region using the F3 statistic revealed signatures of introgression from *Cx. quinquefasciatus*. Within this cluster, a group of cytochrome P450 genes—known to play key roles in detoxification and insecticide resistance—exhibited elevated read depth relative to the genomic background, suggesting a potential copy number variant (CNV). Read depth, and thus inferred gene copy number, increased with maximum summer temperature in populations from BW. However, the CNV was also detected in the northern *Cx. pipiens* reference population, indicating that copy number variation was not necessarily the result of adaptive introgression. In BW populations, consensus sequences from the genes in this CNV region were more similar to the *Cx. quinquefasciatus* reference than to *Cx. pipiens* in Washington, DC, with similarity decreasing northward. Together, these results suggest that populations in warmer environments may harbor amplified insecticide resistance genes introgressed from *Cx. quinquefasciatus*. This pattern is consistent with climate-driven gene flow facilitating the spread of adaptive variation, with potential consequences for vector control and West Nile virus transmission dynamics in the region.

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MOLECULAR ANALYSIS OF INSECTICIDE RESISTANCE IN MOSQUITOES IN NORTHERN GHANA

Rindaline Pokuaa Adu, Bismark Sarkodie Acheampong Adu
University of Sikills Training and Entrepreneurial Development, Kumasi, Ghana

This study examined the molecular basis of insecticide resistance in mosquito populations in Northern Ghana. A cross-sectional design was used. Mosquitoes were collected from urban, peri-urban, and rural areas across the Northern, Savannah, and Upper East regions. A total of 240 adult mosquitoes were sampled and identified using both morphological keys and PCR confirmation. Genomic DNA was extracted from each sample. PCR assays were used to detect resistance genes, including cytochrome P450, glutathione S-transferase (GST), and voltage-gated sodium channel (VGSC). Knockdown resistance (kdr) mutations were also screened. Gene expression levels were measured using quantitative PCR. The results showed that 168 (70.0%) mosquitoes carried at least one resistance marker. Cytochrome P450 genes were overexpressed in 126 (52.5%) samples. kdr mutations were detected in 108 (45.0%) mosquitoes. GST genes were elevated in 92 (38.3%) samples. Multiple resistance markers were found in 74 (30.8%) mosquitoes, indicating combined resistance. Urban populations showed higher resistance levels (78.5%) compared to peri-urban (69.2%) and rural populations (61.3%). Statistical analysis showed that insecticide exposure was strongly associated with resistance (OR = 3.8, 95% CI: 2.1–6.9, $p < 0.001$). Gene expression levels were significantly higher in resistant populations ($p < 0.01$). These findings provide strong empirical evidence that insecticide resistance is widespread in Northern Ghana. Continuous monitoring is needed to guide effective and sustainable vector control strategies.

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COINFECTION OF DENGUE FEVER AND CHIKUNGUNYA VIRUSES IN SELECTED HEALTH FACILITIES IN GHANA

Roberta Dedei Afi Tackie
Noguchi Memorial Institute for Medical Research, Legon, Ghana

Dengue fever and Chikungunya are transmitted through a bite of an infected mosquito, specifically an Aedes aegypti or an Aedes albopictus. The two diseases are not the same, despite having comparable vector species and modes of transmission. Chikungunya is caused by the Chikungunya virus, a member of the Togaviridae family while Dengue fever is caused by the Dengue virus in the Flavivirus family. In areas where both circulate, they can be transmitted together and the early detection of co-infection of both diseases can help prevent complication. This study sought to describe the exposure levels to Dengue fever and Chikungunya viruses among individuals presenting with febrile illnesses. Blood samples were taken from febrile patients who met the standard case definitions for both Dengue fever and Chikungunya and having Malaria ruled out. The samples were obtained from selected health facilities across the country. Serum obtained from the blood samples were used to establish the sero-prevalence of the viral agents with an ELISA assay (Abcam IgG, IgM Elisa kits). Out of the 1080 sera screened from 2016 to 2018, the health facilities in the Northern region recorded the highest positivity of 30.23%, which manifested in pronounced symptomatology and in some instances, severe disease. The Greater Accra region followed with 25.97% and Ashanti region (25.65%) then Western region (33.33) and Volta region (14.29%). Health facilities in the Upper west however had no co-infections. In total, 2016 had the highest positivity rate which was 29.12% followed by 2017 (28.01%) and finally 2018 (13%). In conclusion, our study highlighted the public health concern about the co-circulating and co-infections of Dengue fever and Chikungunya viruses, as well as added to scientific body of knowledge the proportions of their sero-prevalence. It is however to be noted that there may be higher risk for critical outcomes in coinfection patients and therefore calls for further studies.

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THE HIDDEN BURDEN OF DENGUE IN PUERTO RICO

Maile B. Thayer[1], Zachary J. Madewell[1], Laura E. Adams[1], Gabriela Paz-Bailey[1], Michael A. Johansson[2]
[1]Centers for Disease Control and Prevention, San Juan, PR, United States, [2]Northeastern University, Boston, MA, United States

Dengue poses a growing public health challenge, particularly in tropical and subtropical regions. Effective decision-making for dengue prevention and control relies on accurate incidence estimates; however, high rates of asymptomatic infections, nonspecific clinical symptoms, limited diagnostic testing, and gaps in routinely collected data complicate estimation of population-level dengue incidence. Leveraging multiple data sources from Puerto Rico, we integrated active and passive surveillance data, community-based cohort surveys, and published estimates to adjust for under-detection of dengue virus infections. We developed a Bayesian framework to characterize and adjust for key sources of underreporting in dengue surveillance, including estimates of symptomatic infections, healthcare-seeking behavior, diagnostic testing and reporting. The final model provided adjustment factors at each stage of the reporting process, including multipliers for individuals with symptomatic infections, those that seek care, and those that are tested and reported to surveillance systems. Underreporting was substantial across all age groups, with variations across age groups, clinical severity, and infection history. Reporting was more likely among children compared to adults, hospitalized compared

to ambulatory cases, and secondary compared to primary infections. Underreporting was driven primarily by the phase between care seeking and laboratory confirmation, followed by stages of symptom development and care seeking. This framework explicitly models each stage of the reporting process while integrating observational, clinical, and community data to quantify dengue under-detection across age groups in Puerto Rico. Estimates that do not account for these adjustments substantially underestimate true dengue incidence. By integrating each stage of the reporting process and incorporating associated uncertainty, our approach provides more accurate dengue incidence estimates to support prevention and control efforts.

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GENETIC CHARACTERIZATION OF INFLUENZA AND SARS-COV-2 VIRUSES IN DOW BENEFICIARIES DURING THE 2025-2026 SEASON

William Gruner[1], Deanna Muehleman[2], Vivian Hogan[1], Chelsea Harrington[1], Kelsey Lanter[3], James Hanson[1], Padraic Fanning[1], Nyree Watts[1], Elizabeth Macias[1], Anthony C. Fries[4]

[1]Defense Centers for Public Health - Dayton, Dayton, OH, United States, [2]USAF, Gainesville, VA, United States, [3]USAFSAM, Dayton, OH, United States, [4]United States Air Force School of Aerospace Medicine, Wright Patterson Afb, OH, United States

The Department of War (DoW) Global Respiratory Pathogen Surveillance Program conducts testing on respiratory specimens from a worldwide network of sentinel sites using PCR-based assays and next-generation sequencing (NGS) to detect and characterize respiratory pathogens. Analyses aid in the annual selection of influenza vaccine strains and help define the impact of influenza and SARS-CoV-2 in the DoW. The program collects respiratory specimens and metadata from DoW active duty and beneficiaries with influenza-like or COVID-19-like illness symptoms across 100+ global sentinel sites. PCR-confirmed influenza and SARS-CoV-2 positive specimens are further characterized by NGS. In combination with partner laboratory data, phylogenetic analyses, and lineage determinations are performed to assess the genetic changes occurring in these viruses. We analyzed 1,300 influenza viruses, including 138 A(H1N1)pdm09, 1,041 A(H3N2), and 121 B/Victoria. Among A(H1N1)pdm09 viruses, ten were clade 5a.2a with 70% subclade C.1.9.3 and 124 were 5a.2a.1 with 56% subclade D.3.1. For A(H3N2), all were clade 2a.3a.1 with 94% subclade K. All B/Victoria viruses were clade V1A.3a.2 with 72% subclade C.3.1. Lineages identified for 365 SARS-CoV-2 specimens included 2 BA.3, 3 BA.3.2.2, 7 JN.1, 1 KP.2, 1 KP.3, 6 KP.3.1.1, 26 LP.8.1, 1 MC.10.1, 49 NB.1.8.1, 2 XEC, 158 XFG, 86 XFG.3, and 23 other recombinant viruses. Influenza activity remained moderate for the 2025-2026 season compared to the previous season. The predominant subclades for each subtype drifted enough from the 2025-2026 vaccine strains that new selections for A(H1N1)pdm09, A(H3N2), and B/Victoria were selected for the 2026-2027 influenza vaccine strain recommendations. SARS-CoV-2 activity remained low like the previous season, with the dominant lineage changing from LP.8.1 to XFG through the season.

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ACTIVE SURVEILLANCE FOR HEARTLAND VIRUS: CLINICAL AND GENOMIC EPIDEMIOLOGY

Diana Zychowski[1], Lauryn Ursery[2], Sophia Sukkestad[3], Alaa Ahmed[3], Dana Giandomenico[2], Shuntai Zhou[2], Melissa Miller[2], Jonathan J. Juliano[2], Anne Piantadosi[3], Ross M. Boyce[2]

[1]Hennepin Healthcare, Minneapolis, MN, United States, [2]University of North Carolina at Chapel Hill, Chapel Hill, NC, United States, [3]Emory University, Atlanta, GA, United States

Heartland virus (HRTV) is an emerging tick-borne virus capable of causing severe illness and death. The burden of disease is likely underestimated due to limited seroprevalence studies, lack of commercially available diagnostic tests, and an overlapping clinical syndrome with more commonly diagnosed bacterial diseases such as spotted fever group rickettsiosis or ehrlichiosis. Infrequent testing during periods of viremia has resulted in a relative scarcity of clinical specimens and subsequent lack of published sequences. Together, this has hindered our understanding of the clinical and viral determinants of HRTV pathogenicity. Active surveillance for Heartland virus disease was conducted at a large academic center from March to September 2024. Enrolled subjects included those who had testing sent for *Ehrlichia* polymerase chain reaction (PCR) along with fever and 2 of the following 3 criteria: leukopenia, thrombocytopenia, and/or elevated liver function tests. Specimens with detectable RNA underwent whole genome sequencing and analysis. Over 800 specimens were received with 53 individuals meeting enrollment criteria. Among these 53, two (3.8%) had detectable HRTV RNA in whole blood during the time of *Ehrlichia* PCR testing. The two cases had disparate clinical manifestations: one had mild disease, which was identified in an outpatient setting, while the second required intensive care unit-level support. Heartland virus genome sequences from the two cases were phylogenetically divergent and more similar to viruses from other states than they were to one another. Genomic conservation with geographic clustering was more common in tick-derived sequences. Despite only two prior reported cases of Heartland virus disease in North Carolina, we identified two individuals with acute HRTV viremia. These results challenge prior assumptions regarding the phylogenomic diversity of Heartland virus, expand current understanding of the clinical spectrum of HRTV disease, and highlight the value of active surveillance for detecting and characterizing emerging tick-borne pathogens.

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APPLYING AND VALIDATING THE WATER-ASSOCIATED DISEASE INDEX (WADI) FOR DENGUE VIRUS VULNERABILITY MAPPING IN PUERTO RICO, 2010-2020

Rafael A. Saavedra Hernandez[1], Grayson Brown[1], Diego Zavala-Zegarra[2], Melissa Marzán-Rodríguez[2]

[1]Puerto Rico Vector Control Unit, San Juan, PR, United States, [2]Ponce Health Science University, Ponce, PR, United States

Dengue virus (DENV 1-4), transmitted primarily by *Aedes aegypti*, caused over 32,000 reported cases across Puerto Rico's 78 municipalities between 2010 and 2020, including major epidemics in 2010 (n=10,967) and 2013 (n=10,351). Passive surveillance consistently underestimates true transmission burden, underscoring the need for prospective vulnerability tools. The Water-Associated Disease Index (WADI), a composite index integrating environmental exposure (temperature, precipitation, land use, population density) and socioeconomic susceptibility determinants of dengue transmission, has been validated in Malaysia, Brazil, Dominica, Vietnam, and Jamaica, but never applied in Puerto Rico or any U.S. jurisdiction. We conducted a descriptive ecological study across all 78 municipalities (2010-2020) to validate the WADI and identify the optimal exposure-to-susceptibility weighting scheme for this context. Three models were evaluated: WADI1 (25%/75%), WADI2 (50%/50%), and WADI3 (75%/25%). Validation combined Spearman correlations, chi-squared tests, Wilcoxon signed-rank tests, negative binomial (NB) and zero-inflated negative binomial (ZINB) regression, and Getis-Ord Gi* spatial hot spot analysis. WADI3 consistently provided the best fit: Spearman r=0.45 (2010-2013 epidemic period), OR=2.82 for dengue-positive municipality-months (95% CI: 1.62-4.93), IRR=10.65 per WADI3 unit in NB regression ($p<0.001$), and lowest AIC in ZINB models (19,905 vs. 20,365 for WADI2). Seven metropolitan dengue hot spot municipalities were confirmed as WADI3 hot spots. Dorado and Toa Alta were identified as WADI3 hot spots despite low historical case counts, emphasizing the need to prioritize these municipalities for dengue vector control by incorporating multidimensional metrics such as WADI, rather than relying solely on historical case data. Exposure factors, climate and population density, are the dominant drivers of spatial dengue transmission risk in Puerto Rico, with implications for arboviral surveillance and vector control prioritization across the Caribbean.

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SUSTAINING NATIONAL MPOX DIAGNOSTICS DURING CONCURRENT OUTBREAKS: SUPERIOR YIELD AND SYSTEM RESILIENCE FROM A MILITARY SENTINEL SURVEILLANCE LABORATORY IN UGANDA

Henry Kyobe Bosa[1], Daniel Bulwadda[2], Ingrid Ampeire[1], Yonah Muganzi[2], Karen Nakiyingi Kyobe[3], Andrew Nsawotebba[4], Ambrose Musinguzi[2]

[1]Ministry of Health, Kampala, Uganda, [2]Rear Public Health Laboratory, Kampala, Uganda, [3]Lira Univeristy, Lira, Uganda, [4]National Health Laboratory & Diagnostic Services, Kampala, Uganda

Concurrent high-consequence outbreaks risk overwhelming centralized laboratory systems, degrading diagnostic continuity and epidemic response. Uganda activated the Rear Public Health Laboratory (RPHL) within a 17-site military-linked sentinel surveillance network to augment national capacity. During the 2025 mpox outbreak, central laboratory resources were partially diverted to the Sudan ebolavirus disease response; we assessed RPHL diagnostic yield relative to the national system. We conducted a retrospective comparative analysis of mpox PCR testing at RPHL and the national laboratory network (NHLDS) from January-December 2025, comparing positivity rates by χ^2 test, estimating effect size as risk difference (95% CI), and assessing temporal dynamics across outbreak phases. RPHL tested 769 suspected cases, confirming 456 infections (positivity 59.3%), compared to 5,880 positives among 10,826 tests nationally (54.3%). RPHL demonstrated a higher diagnostic yield (risk difference +5.0%, 95% CI +1.4% to +8.6%, p=0.007). Early outbreak amplification was more pronounced at RPHL, peaking in March (76.9%) and April (58.1%), exceeding national trends and indicating preferential capture of high-transmission clusters. Both systems converged to near-zero detection from August onward, consistent with epidemic control. Critically, in the same period, RPHL sustained up to 13% of national testing volume and maintained uninterrupted throughput during diversion of central laboratory capacity. RPHL did not merely backstop national diagnostics; it outperformed centralized testing in yield while sustaining system continuity under stress. Higher positivity and earlier peak detection suggest enhanced case targeting and accelerated epidemic signal capture. Decentralized, sentinel surveillance-linked laboratories offer dual advantage: surge capacity and superior epidemiologic resolution. Embedding such military-civilian platforms within national systems, integrated with genomic surveillance, represents a strategic imperative for resilience against overlapping epidemics in resource-constrained settings.

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EPIDEMIOLOGY OF HUMAN PARAINFLUENZA VIRUSES AMONG COMMUNITY-BASED PEDIATRIC OUTPATIENTS UNDER FIVE YEARS OF AGE WITH ACUTE RESPIRATORY INFECTIONS IN BILIRAN, PHILIPPINES, 2014-2020

Yukito Nakanishi[1], **Yusuke Sayama**[1], Mayuko Saito[1], Michiko Okamoto[1], Takeaki Imamura[1], Mariko Saito-Obata[1], Socorro P Lupisan[2], Hitoshi Oshitani[1]

[1]Tohoku University of Graduate School of Medicine, Sendai, Japan, [2]Research Institute for Tropical Medicine, Manila, Philippines

Human parainfluenza viruses (HPIVs) are a major cause of acute respiratory infections (ARIs) in children and are classified into four types (HPIV-1 to HPIV-4) by serological analysis. However, long-term epidemiological data from tropical community-based outpatient settings remain limited. We investigated the epidemiology, type distribution, age patterns, and reinfection of HPIVs among children under 5 years with ARIs in Biliran, Philippines. From March 2014 to March 2020, outpatients aged under 5 years with ARIs at a public primary health unit were enrolled. Nasal or nasopharyngeal swabs and demographic data were collected. Samples were screened for HPIV-1 to HPIV-4 by type-specific RT-PCR and by Sanger sequencing. Repeated detection of the same HPIV type in the same child was considered reinfection, and paired strains were analyzed for the two viral surface proteins, the Fusion (F) and Hemagglutinin-Neuraminidase (HN) genes. Among 4,155 samples from 1,484 children, 384 (9.2%) were positive for HPIV. HPIV-3 was the most frequently detected type (n=176, 45.8%) in HPIV-positive cases, followed by HPIV-4 (n=88, 22.9%), HPIV-1 (n=82, 21.4%), and HPIV-2 (n=38, 9.9%). HPIV-3 infection was detected in 40 of the 73 months covered by this study. Children with HPIV-3 infection had the lowest median age (16 months), followed by those with HPIV-1 (19 months), HPIV-4 (20 months), and HPIV-2 (27.5 months), and were significantly younger than those infected with HPIV-2 (p = 0.001). Reinfection with only HPIV-3 was identified in five children. F and HN sequences of the HPIV-3 showed 1-12 amino acid substitutions between primary and reinfection strains. HPIV contributed substantially to the burden of pediatric outpatient ARIs in this tropical community. In particular, many cases of HPIV-3 infection were observed among young children, and cases of reinfection were also confirmed. These findings provide baseline data on HPIV epidemiology in a tropical community-based outpatient setting and support the value of longitudinal outpatient surveillance for understanding type-specific epidemiology.

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DENGUE MORTALITY IN PUERTO RICO DURING THE 2024-2025 OUTBREAK

Liliana Sánchez-González[1], Yamila Romer[1], Jomil Torres-Aponte[2], Aidsa Rivera[1], Dania M. Rodriguez[1], Iris Cardona[2], Miriam Ramos[2], Ruby Serrano-Rodriguez[2], Melissa Marzan-Rodriguez[1], Gabriela Paz-Bailey[1], Laura Adams[1], Miguel Valencia-Prado[2]

[1]CDC Dengue Branch, San Juan, PR, United States, [2]Puerto Rico Department of Health, San Juan, Puerto Rico

Dengue, a mosquito-borne viral febrile illness, is endemic in Puerto Rico (PR). After several years of low incidence, cases started to increase in late 2023, and a dengue outbreak was declared on March 25, 2024. Dengue can progress to severe disease or death, with severe clinical manifestations including severe plasma leakage, bleeding, and organ impairment. We analyzed cases from the Puerto Rico Department of Health (PRDH) dengue surveillance system and included cases with positive results for dengue virus (DENV) by RT-PCR or IgM. We analyzed demographic data and case fatality rates (CFR) by age group, and reviewed medical records, when available, to identify the clinical manifestations of severe disease among fatal cases. During Jan 2024-Dec 2025, 9,656 dengue cases were reported in PR. Among them, 5,033 (52%) were hospitalized and 405 (4%) had severe dengue. A total of 12 dengue-associated deaths were identified; 6 (50%) were female, and median age was 76 years (range 17-90). The CFR was 0.12% among all ages, but highest (6/214, 2.8%) among patients >80 years old (p<0.001). The health region with most reported fatal cases was San Juan (n=6, 50%). DENV-3 was identified in 8 (67%) fatal cases, with DENV-2 in 2 (17%) cases, and 2 (17%) only positive by IgM. Detailed clinical information was available for eight fatal cases. The most frequently identified severe clinical presentation (n=4, 50%) was shock and/or severe plasma leakage, with severe organ involvement in the other patients including hepatitis (n=2, 25%), encephalitis (n=1, 12.5%), and myocarditis (n=1, 12.5%). Death occurred within three days of hospitalization in 4 (50%) patients, and the median hospital stay was 5 days (range 1-22). During the 2024-2025 outbreak in PR, dengue-associated mortality was disproportionally high in elderly patients. Shock was the most common severe dengue clinical presentation identified among fatal cases, highlighting that severe plasma leakage is the greatest risk among dengue patients of all ages. Regular refresher trainings for clinicians emphasizing the need for close monitoring and timely use of IV fluids may help improve dengue patients' outcomes.

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INCREASING DENGUE BURDEN AMONG ADULTS: SHIFTS IN GLOBAL DENGUE EPIDEMIOLOGY, 1990-2025

Yamila Romer[1], Zachary Madewell[2], João Bosco Siqueira Junior[3], Yaritbel Torres-Mendoza[4], Laura Adams[5], Gabriela Paz-Bailey[6]

[1]Center for Disease Control and Prevention, Atlanta, GA, United States, [2]San Juan, PR, United States, [3]Sanofi Brazil, Goiania, Brazil, [4]CDC, Lafayette, IN, United States, [5]CDC, San Juan, PR, United States, [6]CDC Dengue Branch, San Juan, PR, United States

Dengue has historically been considered a pediatric disease, but evidence suggests an increasing proportion among adults. We assessed temporal changes in dengue age distribution using global modelling estimates and surveillance data from two countries in the Americas. We analyzed Global Burden of Disease 2023 estimates (1990–2023); Brazil surveillance data (2005–2025); and U.S. ArboNET data (2010–2025). We examined temporal changes in age composition using descriptive analyses and generalized additive models, stratified globally by morbidity and mortality, by region in Brazil, and by transmission setting in the U.S. Globally, the annual number of dengue cases increased from 2.93 million in 1990 to 37.48 million in 2023. The proportion of dengue burden among adults aged >50 years increased from 13% to 24% of cases, 6% to 20% of disability-adjusted life years (DALYs), and 16% to 42% of deaths. The share among those aged <15 years declined from 37% to 25% for incidence, 74% to 40% for DALYs, and 62% to 25% for deaths. In Brazil, the proportion aged ≥50 years increased from 16.5% in 2005–2009 to 25.4% in 2021–2025, with largest increases in the Southeast (+7.7 percentage points) and South (+6.7 percentage points). In ArboNET, the proportion aged ≥50 years changed modestly in endemic jurisdictions (17.6% in 2010–2014 to 20.5% in 2020–2025) and more in non-endemic jurisdictions (36.8% to 45.4%). These findings indicate a sustained shift in the age distribution of dengue burden toward older populations. This shift has implications for dengue programs, as surveillance systems, clinical management, and prevention have historically emphasized pediatric risk and may not adequately address older populations.

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ASSESSING THE SPATIOTEMPORAL TRANSMISSION INTENSITY OF DENGUE VIRUSES IN TAIWAN

Yao Chang[1], Gabriel Ribeiro dos Santos[2], Chih Lin[1], Nien-Kung Li[1], Yu-An Li[1], Chung-Hao Huang[3], Henrik Salje[4], Cheng-Sheng Lee[1], Hsiao-Han Chang[1]

[1]National Tsing Hua University, Hsinchu, Taiwan, [2]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [3]Kaohsiung Medical University, Kaohsiung, Taiwan, [4]University of Cambridge, Cambridge, United Kingdom

Dengue is a mosquito-borne virus causing recurrent outbreaks in Taiwan. Reported cases often underestimate true transmission dynamics due to asymptomatic infections and underreporting. Spatial mismatches between infection and reported case locations can further bias estimates of transmission risk. To improve inference of spatiotemporal transmission risk, we analyzed 6,418 Taiwan Biobank samples collected between 2008 and 2021 across all cities and determined seropositivity for dengue IgG antibodies using ELISA, with an overall seroprevalence of 4.1% (95% CI: 3.6-4.6%). Using a Bayesian spatially explicit regression framework based on integrated nested Laplace approximations (INLA), we estimated dengue force of infection (FOI) for 349 townships in Taiwan, accounting for Aedes mosquito abundance, population density, and human mobility. On average, dengue outbreaks infected 0.3% of the susceptible population, reaching up to 0.5% in southern Taiwan. We then reconstructed seroprevalence and estimated the number of infections in the population. While the proportion of seropositive individuals was low in Taipei City, the estimated number of infections remained high due to its large population. Comparing model predictions with reported cases, we estimated reporting rates and found substantial spatial and temporal heterogeneity. The overall reporting rate was 1.6% (95% CI: 1.3-1.8%), increasing to 5.7% (95% CI: 4.4-6.9%) during larger outbreaks and decreasing to 0.37% (95% CI: 0.3-0.4%) in non-outbreak years, likely reflecting enhanced surveillance. Underreporting persisted in smaller towns (<16,000 residents) across years, which reported only 0.95% of expected cases. Age-stratified analyses showed lower reporting rates in children (<14 years; 1.6%) and a gradual increase with age, particularly in those over 50 years (5.1%). Overall, this study demonstrates the value of large-scale serological data for reconstructing dengue FOI and quantifying spatial heterogeneity in transmission and reporting, providing insights to support more targeted public health interventions.

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WASTEWATER-BASED EPIDEMIOLOGY OF DENGUE VIRUS FOR EARLY OUTBREAK DETECTION IN THE PERUVIAN CENTRAL RAINFOREST

Cesar A. Valdivia-Carrera[1], Daniel A. Andrade[2], Brenda L. Gonzales[2], Jessica Fernandez-Roman[3], Santos S. Narro-Lopez[3], Luis A. Gomez-Puerta[2], Faride Altamirano[2], Cesar M. Gavidia[2], Ana C. Ho-Palma[4], Miguel M. Cabada[5], Marta Rusiñol[6], Eloy Gonzales-Gustavson[2]

[1]Facultad de Medicina, Universidad Nacional Mayor de San Marcos, Lima, Peru, [2]Facultad de Medicina Veterinaria, Universidad Nacional Mayor de San Marcos, Lima, Peru, [3]Hospital Regional Docente de Medicina Tropical "Dr. Julio Cesar Demarini Caro", Junin, Peru, [4]Facultad de Medicina, Universidad Nacional del Centro del Peru, Junin, Peru, [5]Facultad de Medicina, Universidad Peruana Cayetano Heredia, Lima, Peru, [6]Facultat de Biologia, Universitat de Barcelona, Barcelona, Spain

Dengue incidence has increased dramatically in recent decades due to climate change, rapid urbanization, and expanding vector distribution in endemic regions. In Peru, conventional surveillance systems are often limited by delays and underreporting, restricting timely outbreak detection. Wastewater-based epidemiology (WBE) has emerged as a promising tool for community-level surveillance. However, its application to dengue remains limited. This study evaluates the feasibility of applying WBE for dengue virus (DENV) surveillance in Chanchamayo, a high-burden urban area in the Peruvian Central Rainforest. Three experimental approaches were conducted prior to field implementation. First, DENV RNA persistence in wastewater was assessed at 4°C, 25°C, and 35°C for 21 days, showing greater stability at lower temperatures, with 90% decay times of 27, 17, and 6 days, respectively. Second, four viral recovery methods were compared, with ultrafiltration achieving the highest recovery efficacy (61%), followed by direct extraction (42%) and polyethylene glycol precipitation (7%), while skimmed milk flocculation remained below the detection limit. Third, electronegative membranes evaluated as a passive sampling strategy demonstrated a recovery efficacy of 20%. Based on these findings, a field study is underway, involving twice-weekly wastewater sampling over a six-month period using two approaches: conventional and passive. Conventional sampling consists of collecting 1-L samples followed by viral concentration using ultrafiltration, while passive sampling involves deploying electronegative membranes submerged in wastewater for 24 hours to adsorb viral particles. DENV RNA is quantified by RT-qPCR, and the results will be correlated with reported dengue cases using 1- and 2-week time lags to assess the potential for early outbreak detection. Furthermore, DENV serotypes will be determined using serotype-specific RT-qPCR. Preliminary experimental results demonstrate the feasibility of detecting and recovering DENV from wastewater, supporting the application of WBE for dengue surveillance.

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MAPPING AND PREDICTING DENGUE SEASONALITY AT SUBNATIONAL LEVELS ACROSS GLOBAL REGIONS

Porcia Manandhar[1], Bernardo García-Carreras[2], Pengcheng Fang[1], Bachir Assao Neino[2], Matthew DT Hitchings[2], Angkana T. Huang[3], Amy Wesolowski[1], Derek AT Cummings[1]

[1]Johns Hopkins University, Baltimore, MD, United States, [2]University of Florida, Gainesville, FL, United States, [3]National University of Singapore, Singapore, Singapore

Dengue is a rapidly spreading, climate-sensitive arboviral disease with strong seasonal and spatial variation. Urbanization and travel have expanded transmission into non-endemic regions globally. Quantifying seasonality at subnational scales and understanding how climatic factors drive incidence and transmission are critical for surveillance and control. Using global historical case data, this study refines weekly- and monthly-scale effective reproductive numbers (R(t)) and characterizes dengue seasonality with predictive models using environmental and climate covariates. We are updating a harmonized dataset of case counts at daily, weekly, and monthly resolutions across administrative levels 1 and 2 units from published and unpublished studies and repositories. For each time series, we estimate R(t) and seasonality metrics including peak R(t) values and their timing. We will fit regression (generalized additive models) and machine-learning models to R(t) estimates and seasonality metrics, with matched environmental and climate records (including temperature, rainfall, and humidity) as predictors. Models will be evaluated by out-of-sample predictive performance to prevent overfitting. We also evaluate effects of temporal and spatial aggregation and of varying climate-based generation times. We will characterize seasonality of transmission across diverse subnational units and quantify its relationship with local temperature and rainfall cycles. Predictive models will generalize these relationships to estimate dengue seasonality globally even in locations lacking reported case or serological data. A web-based tool providing location-specific R(t) estimates will be built alongside our models. Understanding when and where dengue transmission peaks is essential for targeting surveillance and predicting risk. By grounding climate-driven predictions in empirical R(t) estimates, this framework can provide seasonality baselines robust to changes in susceptibility and estimable in data-limited settings.

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GENOMIC CHARACTERIZATION OF HUMAN ADENOVIRUSES IN PATIENTS PRESENTING WITH FEBRILE AND RESPIRATORY INFECTIONS AT SELECT HOSPITALS IN KENYA

Ann Cheptoo Laigong[1], Gathii Kimita[2], Samoel Ashimosi Khamadi[3], Steven Ger Nyanjom[4], John Njenga Waitumbi[2]

[1]Kenya Medical Research Institute Graduate School/Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya, [2]Walter Reed Army Institute of Research-Africa, Kisumu, Kenya, [3]Kenya Medical Research Institute – Center for Viral Research, Nairobi, Kenya, [4]Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya

Human adenoviruses (HAdVs) cause respiratory illness especially in children and the immunocompromised. Clinical manifestation varies by HAdV genotypes, yet genomic data, critical for genotyping, from Kenya is limited. We determined the diversity of HAdV and assessed recombination in sequence data obtained from patients presenting with febrile and respiratory illnesses in six sentinel surveillance hospitals in Kenya. DNA from nasopharyngeal swabs (Aug 2024–Jan 2026) was extracted and used for sequence library preparation and subsequent viral enrichment using the VirCapSeq-VERT probe set designed at the Center for Infection and Immunity, Columbia University. Enriched libraries were sequenced on the NextSeq 2000 (Illumina, San Diego, CA, USA). Following genome assembly of HAdV, genotyping was done using capsid genes (penton, hexon and fiber) and whole genome phylogeny. Recombination was assessed using Recombination Detection Program (RDPv5). Of 1969 nasal samples, 163 (8.3%) had HAdV reads, of which, 58 (35.6%) were resolved to B (n=23) and C (n=35) species. Of the 23 HAdV-B, capsid-based typing identified 22 as B66 (n=12) and B114 (n=10). By whole-genome phylogeny, all B66 genotypes clustered with B7, while B114 genotypes split between B3 (n=6) and B114 (n=4). Of the 35 HAdV-C only 7 genotypes could be confidently called: capsid typing and whole genome identified C89 genotype (n=5) while two were discordant, clustering with C5 on penton but C89 on hexon, fiber, and whole genome. A recombinant genome between B114 and B7 ($p<0.05$) supported by all 7 algorithms in RDP5 was, with a hexon breakpoint was identified. To our knowledge, this is the first report on the HAdV genotype diversity in patients with febrile and respiratory infections in Kenya. Discordance between capsid-based typing and whole-genome phylogeny highlights the limitations of single gene-based typing and supports genome-based approaches. Recombination highlights ongoing viral evolution, emphasizing the need for continued genomic surveillance.

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GENETIC DIVERSITY AND LINEAGE DYNAMICS OF DENGUE VIRUS IN THE PERUVIAN AMAZON

Xiomara Merino Merino[1], **Francesca Falconi-Agapito**[1], Jaide Sierra-Aroni[1], Fiorella Torres[2], Carlos Villa[2], César Ramal Asayag[3], Michael Talledo[1], Kevin K. Ariën[4], Philippe Selhorst[4]

[1]Universidad Peruana Cayetano Heredia, Instituto de Medicina Tropical Alexander von Humboldt, Lima, Peru, [2]Hospital Santa Gema, Yurimaguas, Peru, [3]Universidad Nacional de la Amazonía Peruana, Iquitos, Peru, [4]Department of Biomedical Sciences, Unit of Virology, Institute of Tropical Medicine, Antwerp, Belgium

Dengue epidemics in Peru have historically been dominated by a single serotype, with periodic serotype replacement over time. Recent concurrent circulation of multiple serotypes in the Peruvian Amazon raises concern for increasing transmission complexity and possible progression toward hyperendemicity. In this context, genomic surveillance provides resolution beyond serotype-specific PCR by identifying lineage turnover and patterns of viral relatedness across time and place.We analyzed dengue virus (DENV) genomes obtained from samples collected between 2018 and 2024 in Yurimaguas and Iquitos, two endemic sites in the Peruvian Amazon. Sequencing was performed using an Oxford Nanopore RNA sequencing workflow for pathogen detection, using CRISPR-Cas9 host depletion and SISPA-based viral enrichment. Preliminary phylogenetic analyses were conducted on the envelope (E) gene. A total of 110 new DENV E gene sequences were analyzed, including DENV-1 (n=13), DENV-2 (n=91), and DENV-3 (n=6). DENV-1, sequences clustered within genotype V and grouped with recent Peruvian isolates, forming a supported subcluster characterized by a consistent amino acid substitution (I457V). DENV-2 showed two well-supported clades, with 2018–2021 sequences clustering within genotype III, and 2023–2024 sequences within genotype II, consistent with a temporal shift in circulating genotypes. DENV-3 sequences, all from Yurimaguas clustered within genotype III and grouped with other recent Peruvian isolates, while older Peruvian sequences (2002–2007) formed a more divergent cluster.These preliminary findings show concurrent circulation of multiple DENV serotypes together with temporal structuring of distinct viral lineages in the Peruvian Amazon. Such patterns may have important epidemiological implications in a region historically shaped by single-serotype epidemics. Phylogeographic and time-resolved analyses at genomic level are ongoing to further reconstruct lineage movement and clarify patterns of viral introduction and spread across the region.

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GENOMIC CHARACTERIZATION OF 104 POWASSAN VIRUS ISOLATES FROM HUMAN-ENCOUNTERED TICKS

Jessica Crooker[1], Saravanan Thangamani[2]

[1]State University of New York Upstate Medical University, Syracuse, NY, United States, [2]SUNY Upstate Medical University, Syracuse, NY, United States

Powassan virus (POWV) is a severe tick-borne virus that can cause a neuroinvasive disease encompassing encephalitis, meningitis, and

meningoencephalitis with a mortality rate of approximately 15%. For those who survive neuroinvasive POWV, 50% experience severe and often permanent neurologic sequalae. Ongoing symptoms experienced by survivors have included paralysis, muscle wasting, aphasia, severe headaches, and many other neurologic symptoms that can have a profound impact on quality of life. Over the past two decades, the number of cases of neuroinvasive POWV infection have increased dramatically, as have case numbers of many other tick-borne diseases. With an increasing number of cases, understanding POWV evolution and how differences in circulating strains of POWV may impact clinical course is essential for developing better diagnostic criteria and strategies towards prevention and treatment. In order to study POWV dynamics of change and understand currently circulating POWV strains, we turned to community-based science and identified using qRT-PCR POWV-positive human-encountered ticks that were submitted to the Upstate Tick Testing Program. Across 2020-2025, we have sequenced 104 human-encountered Powassan-positive ticks from the northeastern United States allowing for insight into circulating isolates of POWV that pose a risk for human infection. POWV isolates from ticks showed variation in both nucleic and amino acid sequences. We have been able to identify sites predicted to be under positive selection in important structural and nonstructural regions; positive selection was predicted within the C, E, NS1, NS2A, NS3, and NS5 protein regions, and in key cleavage sites like that between C and prM. Through detailed geographic and temporal data of when and where ticks were encountered, we are also able to better understand how POWV is changing across time and space, specifically in human-encountered ticks. This work can help to identify important POWV dynamics and identify emerging strains of POWV that may pose increasing risk to human health.

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DIAGNOSTIC PERFORMANCE AND VIRAL LOAD DYNAMICS OF MPXV CLADE IIB IN AN HIV-PREVALENT COHORT USING A FIELD-DEPLOYABLE INSULATED ISOTHERMAL PCR (IIPCR) PLATFORM

Chi-chun Tsai[1], Ping-Chang Lin[2], Ching-Yi Tsai[2], Shang-Yi Lin[3]
[1]Tropical Medicine Center, Division of Infectious Diseases, Kaohsiung Medical University Hospital, Kaohsiung City, Taiwan, [2]Tropical Medicine Center, Kaohsiung Medical University Hospital, Kaohsiung City, Taiwan, [3]Division of Infectious Diseases, Kaohsiung Medical University Hospital, Kaohsiung City, Taiwan

Since 2022, the global outbreak of Mpox caused by the Mpox virus (MPXV) Clade IIb has underscored the need for timely and decentralized molecular diagnostics. Although quantitative real-time PCR (qPCR) remains the diagnostic gold standard, its infrastructure requirements may limit accessibility in resource-constrained settings. Insulated isothermal PCR (iiPCR) represents a potential field-deployable alternative; however, clinical validation data for MPXV remains limited. We prospectively enrolled 24 qPCR-confirmed Mpox patients between 2023 and 2024. Multiple specimen types were tested using the POCKIT Central MPXV iiPCR system and reference qPCR. Analytical sensitivity was assessed using serial viral dilutions. Viral load dynamics were evaluated using cycle threshold (Ct) values across specimen types and days post-symptom onset. Phylogenetic characterization was performed using a four-gene Sanger sequencing approach. Both iiPCR and qPCR demonstrated identical analytical limits of detection at 10^{-1} PFU/mL and complete qualitative concordance in cultured samples. Lesion-derived specimens showed the highest detection rates (100%) and consistently lower Ct values, whereas non-lesion specimens exhibited lower and more variable positivity. No clear differences in viral load-based diagnostic performance were observed between HIV-positive patients receiving antiretroviral therapy (ART) and HIV-negative patients. Phylogenetic analysis confirmed exclusive circulation of MPXV Clade IIb. Under the conditions evaluated, the POCKIT Central iiPCR system achieved diagnostic performance comparable to qPCR, particularly for lesion-derived specimens. In this ART-treated HIV-prevalent cohort with preserved immune function, HIV co-infection was not associated with differences in diagnostic performance, supporting the potential utility of iiPCR for decentralized Mpox testing.

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GENOME CHARACTERIZATION AND PHYLOGENETIC ANALYSIS OF CRIMEAN-CONGO HEMORRHAGIC FEVER VIRUS FROM RHIPICEPHALUS EVERTSI EVERTSI TICKS IN NAIROBI CITY, KENYA

Jane Wambui Thiiru[1], Gladys C. Kerich[1], JANET Ambale[1], Santos J. Yalwala[1], David Ochieng Oullo[1], Nicholas Odemba[1], Ngugi Jacob[1], Lucy Isabella[1], Cynthia Ojino[1], Paul Kyalo[1], Gathii Kimita[2], Elizabeth Kioko[1], Joel Lutomiah[1], John Eads[1]
[1]Walter Reed Army Institute of Research-Africa, Kisumu, Kenya, [2]KEMRI-MRD-K, Kisumu, Kenya

Crimean-Congo Hemorrhagic Fever (CCHF) is a severe zoonotic disease with case-fatality rates exceeding 30%. It is caused by CCHF virus (CCHFV), a tick-borne pathogen primarily associated with Hyalomma Spp. Despite its endemicity in East Africa, genomic data from the region, particularly Kenya, remains limited. Entomological surveillance in areas of wildlife-livestock-human interaction is critical for identifying virus circulation and assessing risk. Entomological investigations were conducted from 24.02-08.03.2026 across four Kenyan counties: Nairobi city, Laikipia, Isiolo, and Samburu. Ticks were collected from livestock and vegetation using grooming and dragging methods, morphologically identified, and pooled. Total RNA was screened for CCHFV using RT-PCR. One positive pool was subjected to target enrichment NGS using VirCapSeq-VERT and sequenced on the Illumina NextSeq 1000. High-quality reads were assembled into consensus genomes, which were used for segment-specific phylogenetic analyses and genome characterization. CCHFV RNA was detected in a pool of Rhipicephalus evertsi evertsi (Ct = 24.0) collected from sheep in Nairobi out of 114 pools tested. Near-complete sequences were obtained for the L (12,045 bp), M (5,347 bp), and S (1,600 bp) segments. L and S clustered within African genotype II, while M clustered within genotype I, closely related to 1950s Ugandan isolates and consistent with established patterns, with no evidence of reassortment. This study provides genomic evidence of CCHFV circulation at a livestock-wildlife-human interface in Nairobi city. The close similarity to 1950s Ugandan strains suggests a naturally circulating, genetically stable lineage consistent with long-term regional persistence or transboundary livestock movement along the cattle corridor. This poses a risk of human infection and outbreaks, as seen in Uganda, that could affect >6M Nairobi residents. These findings highlight the value of integrated entomological and genomic surveillance for public health risk assessment in endemic settings and new areas with demonstrated risk of transmission such as Nairobi city.

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A NOVEL VIRAL METAGENOMIC APPROACH FOR GENOMIC CHARACTERIZATION OF DENGUE AND CHIKUNGUNYA CASES IN PAKISTAN.

Najeeha Talat Iqbal[1], Kumail Ahmed[1], Wajiha Saif[1], Ali Raza Bukhari[1], Hassan Ghayas[2], Erum Khan[3], Wesley C. Van Voorhis[4]
[1]Aga Khan University, Karachi, Pakistan, [2]Karachi, SD, Pakistan, [3]Aga Khan Univ, Karachi, Pakistan, [4]University of Washington, Seattle, WA, United States

Dengue virus (DENV) and Chikungunya virus (CHIKV) are co-circulating arboviruses in Pakistan. In 2022, the highest number of DENV cases was reported in Pakistan, with 32% of cases reported in Sindh province. Our surveillance study identified a total of 291 DENV PCR-positive cases; of those, 44 had mixed serotypes. In 2024, a CHIKV outbreak was reported with a significant decline in DENV cases. Of 91 CHIKV cases, only 32% were PCR positive. The study aimed to confirm (i) mixed serotypes of DENV and (ii) PCR-negative CHIKV and DENV cases using Oxford Nanopore Technology (ONT). A subset of 35 samples, including DENV and CHIKV were selected for viral metagenomics using selection criteria of Ct value < 38 for DENV mixed serotype (n=8), CHIKV positive (n=5), and PCR negative samples (n=22). We employed a viral metagenomic approach using Twist Bioscience Arbovirus research panel on the ONT platform. The viral panel was initially optimized for short-read which was modified for long-read

sequencing compatible with ONT platform. The sample libraries were generated using the Twist Library preparation kit, followed by the target hybridization enrichment workflow. The sequence data were analyzed using Genome Detective, Pan-viral pipeline. The resulting consensus sequences were aligned using MAFFT, and a phylogenetic tree was visualized and annotated using IQTree. The samples with low genome coverage were not included in the analyses. Of mixed serotype, 7/8 were correctly identified as DENV 1 serotype (Subtype IIIA). In DENV PCR negative, 2/17 were DENV-2 serotype (Subtype II.A), and 1/17 was DENV-1 serotype (Subtype III.A). Of the CHIKV PCR-positive samples, 4/5 were confirmed as CHIKV belonging to the East/Central/South African (ECSA) subtype, while 1/5 was identified as DENV2 (Subtype II.A). In CHIKV-PCR-negative cases, 2/5 were confirmed as CHIKV (ECSA subtype), and 1/5 was identified as DENV2 (Subtype II.A). We have successfully developed and adapted Twist Biosciences' protocol for viral detection, demonstrating its effectiveness in resolving mixed infections through long-read metagenomics.

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GENOMIC SURVEILLANCE OF DENGUE VIRUS IN SAUDI ARABIA AND OMAN REVEALS SHIFTING SEROTYPE DYNAMICS AND CROSS-BORDER TRANSMISSION, 2022-2024

Lamiae Boualla[1], Sabir Adroub[1], Rohit satyam[1], Reem Bin Suaydan[2], Asma Al Balushi[3], Fatma Ba Alawi[3], Eitizaz Abdulshakour Zaki[4], Azza Said Al Qayoudhi[3], Roua Alsubki[5], Waleed Al Salem[6], Arnab Pain[7]

[1]King Abdullah University of Science and Tachnology, KAUST, Saudi Arabia, [2]Department of Clinical Laboratory Sciences College of Applied Medical Sciences King Saud University, Riyad, Saudi Arabia, [3]Sultan Qaboos University Hospital, Muskat, Oman, [4]Ministry of Health, Jeddah, Saudi Arabia, [5]king saud university, Riyad, Saudi Arabia, [6]Ministry of Environment, Water and Agriculture of Saudi Arabia, Riyad, Saudi Arabia, [7]King Abdullah University of Science and Technology (KAUST), Pathogen Genomics La, Jeddah, Saudi Arabia

Dengue poses a growing public health threat in endemic and emerging regions, a situation aggravated by climate change, and recent outbreaks in Saudi Arabia and Oman highlight this concern in the Arabian Peninsula. We conducted a large-scale genomic surveillance study to characterise dengue virus (DENV) diversity and transmission dynamics in both countries during the 2022-2024 seasons. In partnership with the national Ministry of Health laboratories in Saudi Arabia and clinicians at Sultan Qaboos University Hospital in Oman, we analysed more than 2,500 DENVpositive serum samples and generated over 2000 viral genomes using longread, ampliconbased Oxford Nanopore sequencing. We developed SeroTyper, a rapid insilico platform for DENV serotyping and genotyping, and compared newly generated genomes with global references to infer serotype distribution, genotype composition and phylogenomic relationships. In Saudi Arabia, we observed a marked shift from a mixed circulation of DENV1 and DENV2 (approximately 60:40) in 2022-2023 to a predominance of DENV2 in 2024, with lowlevel DENV3 detection and no evidence of DENV4 throughout the study period. In Oman, infections during the 2022-2023 outbreak were dominated by DENV2 genotype IIF1.1, while DENV1 and DENV3 were detected for the first time, expanding the known serotype repertoire in the country. Phylogenomic analyses revealed signatures of local persistence as well as likely crosscountry transmission events, and identified characteristic polymorphisms, including multiple nonsynonymous mutations, in key viral proteins such as NS5, NS1 and E. To our knowledge, this is the first largescale genomic study from the MENA region to document contemporary DENV lineages and transmission patterns in Saudi Arabia and Oman, underscoring the value of realtime genomics to inform dengue surveillance and control strategies.

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ESTIMATING MPOX TRANSMISSIBILITY IN ENDEMIC SETTINGS USING APOBEC3 GENOMIC SIGNATURES

Santiago Cardenas, James Lloyd-Smith

University of California, Los Angeles, Los Angeles, CA, United States

Recent mpox outbreaks highlight the need for better understanding of its transmission in endemic settings, but heterogeneity in surveillance and data quality present formidable challenges. Monkeypox virus (MPXV) genome sequencing from mpox cases is becoming increasingly common, enabling detection of APOBEC3 editing signatures, an indicator of sustained human-to-human transmission. As of yet, APOBEC3-type mutation counts in datasets have been used to draw qualitative conclusions about mpox spread; however, the distribution of these signatures has the potential to yield quantitative insights into transmission dynamics, including the reproduction number and degree of superspreading. In this study, we model the accumulation of APOBEC3-type mutations in endemic mpox settings and develop inference tools to estimate transmission parameters from sampled MPXV genomes. Mpox is modeled as a subcritical branching process seeded by repeated spillover. Spillover cases are assumed to have a baseline number of APOBEC3-type mutations, with each transmission event conferring a probability of accumulating an additional mutation. Because case detection for mpox is often clustered due to contact tracing or study design, we incorporate a sampling layer into the model. From a cross-sectional sample of MPXV genomes, yielding a distribution of numbers of host-induced mutations observed across sampled infections, we propose two inference methods: a maximum likelihood estimate for the reproduction number, and a hierarchical Bayesian inference technique for the reproduction number and degree of overdispersion. We test these pipelines on simulated data to assess power, provide guidance on minimum case counts required for reliable inference, and characterize how sampling bias affects parameter estimation. Finally, we test our methods on MPXV genomes from Central Africa collected in 2025. These tools contribute to the repertoire of quantitative techniques that leverage genomic surveillance data to estimate transmission parameters, here designed for application in endemic mpox settings.

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INVESTIGATING GENETIC DIVERSITY OF DENGUE VIRUS IN MATO GROSSO DO SUL FOLLOWING IMPLEMENTATION OF WOLBACHIA-INFECTED MOSQUITO RELEASE.

Sindiso Nyathi[1], Marco Puga[2], Paulo Cesar Pereira dos Santos[3], Crhistinne Gonçalves[4], Jason R. Andrews[5], Derek Cummings[6], Albert Icksang Ko[7], Ricardo Khouri[8], Julio Croda[9]

[1]Yale University, New Haven, CT, United States, [2]Fiocruz Mato Grosso do Sul, Fundação Oswaldo Cruz, Campo Grande, Brazil, [3]Universidade Federal de Mato Grosso do Sul, Campo Grande, Brazil, [4]Faculdade de Medicina, Universidade Federal de Mato Grosso do Sul, Campo Grande, Brazil, [5]Stanford University, Stanford, CA, United States, [6]Johns Hopkins University, Baltimore, MD, United States, [7]Yale School of Public Health, New Haven, CT, United States, [8]Fiocruz, Salvador, BA, Brazil, [9]Oswaldo Cruz Foundation, Mato Grosso do Sul, Campo Grande, MS, Brazil

Wolbachia-based vector control has been shown to be effective at reducing dengue infection risk and is being rolled out in several locations in Brazil. Despite the efficacy of this intervention in controlled trials, where it reduced dengue incidence by 63%, it remains unclear whether dengue virus adaptation poses a threat to the intervention's long-term effectiveness. In this study, we investigate whether genetic diversity of dengue virus populations changes following implementation of mosquito population replacement via *Wolbachia*-based vector control in the Brazilian state of Mato Grosso do Sul. We leverage data from a long-term genomic surveillance program in Mato Grosso do Sul, where *Wolbachia*-infected mosquitoes were successfully released in the city of Campo Grande from 2020 to 2023. As part of the National Arbovirus Sequencing Network Initiative, dengue positive isolates were retrieved from patients with dengue infection presenting to health units, clinics and hospitals in the state, and sequenced. Sample metadata including city, age, sex and collection date

were also obtained. Phylogenetic trees were used to investigate genetic clustering of isolates collected before and after the intervention. 661 newly collected dengue virus sequences from samples collected in Campo Grande, Dourados and other locations were included in the analysis. 280 newly sequenced isolates were typed as dengue 1, 359 were dengue 2, and 22 were dengue 3. An additional 253 publicly available sequences from Mato Grosso do Sul were retrieved from Genbank. This analysis leverages sequencing data collected from Campo Grande, a city in which *Wolbachia*-infected mosquito releases were conducted, and other locations in the state of Mato Grosso do Sul to investigate whether dengue virus populations adapt to this intervention. As *Wolbachia*-infected mosquito releases continue it will be vital to detect dengue adaptation to this intervention in order to develop countermeasures and maintain its effectiveness.

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ANALYSIS OF THE IMPACT OF INOCULATION ROUTE ON VIREMIA, CLINICAL PARAMETERS AND IMMUNOGENICITY IN *AOTUS NANCYMAAE* NON-HUMAN PRIMATE MODEL OF DENGUE VIRUS

Julio A. Ventocilla[1], Lorena L. Tapia[1], Diana P. Cedamanos[1], Macarena Danielli Vittet[1], Hugo Oswaldo Valdivia[2], **Bret Nicholas Peterson**[1], Berlin Londono[3], Brandon K. Wilder[4]

[1]NAMRU SOUTH, Lima, Peru, [2]U.S Naval Medical Research Unit SOUTH, Lima, Peru, [3]New Orleans, LA, United States, [4]OHSU Vaccine and Gene Therapy Institute/NAMRU6, Beaverton, OR, United States

Dengue virus (DENV) infects 100-400 million people worldwide, resulting in 25,000 deaths annually. Suitable animal models for testing novel vaccines and therapeutics are limited and key aspects such as infection kinetics, symptomatology, and route of infection are not standardized. It was shown previously that the intravenous inoculation route develops an asymptomatic disease. Here, we assessed the impact of disparate delivery infection routes on viremia, clinical parameters and immunogenicity in *Aotus nancymaae* non-human primate model of DENV.Groups of *Aotus nancymaae* with DENV serotype 2 (D2S10 strain) were infected using two inoculation routes, intravenous (IV, n=8) and intradermal (ID, n=7) at higher (10^7 plaque-forming units; PFUs) and lower (10^5 PFUs) doses. Whole blood and serum samples were collected at days 0, 4, 8, 12, and 28 post-infection, and RNAnemia, complete blood count (CBC), biochemical markers, and IgG antibodies as key signs of immunogenicity against DENV were measured.Our results showed a prevalence of positive RNAnemia by qPCR in IV and ID routes at day 4 (8/8 vs 7/7), day 8 (5/8 vs 5/7), day 12 (3/8 vs 4/7), and day 28 (0 vs 0). CBC showed a decrease of neutrophils, hematocrit, and platelets at day 4 post-infection with a progressive recovery to normal values starting from day 12 post-infection in both delivery routes. At day 4 post-infection, biochemical analysis showed an increase of ALT, AST, glucose, and tCO2 compared to the baseline. There was an increase of IgG antibodies between day 0 vs 28 post-infection with a trend of elevated antibodies IgG levels being observed in groups with higher infectious doses.Our study showed that *Aotus nancymaae* monkeys are susceptible to experimental DENV infection through intravenous or intradermal delivery routes, producing clinical manifestations during the course of infection similar to those observed in humans. This model supports pre-clinical testing efficacy of novel prophylactics and vaccines against DENV infection.

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EVALUATION OF THE UID ANYCAGE LITE SYSTEM FOR REAL-TIME BODY TEMPERATURE MONITORING AS A CLINICAL READOUT IN DENGUE-INFECTED AG129 MICE

Medha Shekhar[1], Fatemeh Bahari[2], Candra Abraham[1], Colin Osborne[1], Anke Harupa-Chung[1]

[1]Novartis Biomedical Research, Emeryville, CA, United States, [2]Novartis Biomedical Research, Cambridge, MA, United States

Dengue fever (DF), a mosquito-borne disease, is a rapidly growing global threat, now endemic in over 100 countries, putting nearly half the world's population at risk. Despite this burden, no approved antiviral therapies exist, and clinical management remains limited to supportive care. The AG129 mouse model (IFN $\alpha/\beta/\gamma$ $R^{-/-}$), widely used for dengue (DENV) research due to its high susceptibility to infection, enables robust virological, pathological, and immunological readouts, making it useful for antiviral efficacy screening. However, the AG129 model is typically used to assess viral load, survival, weight loss, and clinical score, and there has been limited investigation into its ability to capture other clinically relevant recovery endpoints such as faster fever clearance. Here, we evaluated body temperature as a potential translational readout in DENVinfected AG129 mice over a 10-day period using RFID technology with the UID AnyCage Lite digital homecage system. Unlike the current commonly used method of rectal thermometry, which requires repeated invasive animal handling and provides only intermittent measuring, this approach enables continuous, stress-minimized temperature monitoring. When compared to naïve control animals, mice infected with $1x10^2$ PFU of dengue strain DENV-2 D2Y98P exhibited significantly elevated body temperatures throughout the 10day study period. Treatment with an investigative antiviral therapy normalized body temperature to levels comparable to naïve controls, with treated mice exhibiting significantly lower body temperatures than untreated infected mice beginning on day 2 postinfection (t test, $p<0.001$). Overall, these results support body temperature, measured via implanted RFID transponder, as a minimally invasive and potentially translational marker of clinical recovery in DENV-infected AG129 mice, thereby enhancing the model's utility for preclinical antiviral evaluation.

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ZIKA VIRUS STRUCTURAL GENES ARE NECESSARY FOR VIRAL SHEDDING IN SEMEN

Taylor Nolen, James Weger-Lucarelli, Nisha Duggal

Virginia Tech, Blacksburg, VA, United States

Zika virus (ZIKV; *Flaviviridae*, *Flavivirus*) is a mosquito-transmitted virus that causes flu-like symptoms in adults but can cause microcephaly in newborns exposed *in utero*. After the virus was introduced to the Americas in 2015, sexual transmission of the virus was reported and was estimated to cause 3-23% of ZIKV cases, with up to 50% of infected men shedding ZIKV in semen. Active replication of ZIKV in men occurs in the epididymis, the site of sperm storage, which contributes to viral shedding in semen. The most closely related virus to ZIKV, Spondweni virus (SPOV), displays poor shedding in semen; however, it is still able to disseminate to the epididymis. Currently, it is unknown what viral genes of ZIKV contribute to sexual transmission of the virus and which epididymal cell types contribute to viral shedding. Here, we investigated the mechanism of ZIKV shedding in semen. Using bacteria-free cloning techniques, we generated chimeric viruses in which prM/E structural genes were swapped between ZIKV and SPOV. Next, we infected male mice with parental and chimeric viruses and collected ejaculates. We found that mice infected with ZIKV containing SPOV prM/E had significantly lower rates of virus in ejaculates (25% positive) compared to ZIKV-infected mouse ejaculates (77% positive). However, mice infected with SPOV containing ZIKV prM/E shed virus in ejaculates similarly to mice infected with SPOV. These results indicate that prM/E genes are necessary, but not sufficient, to cause viral shedding in ejaculates. Future studies with these chimeras will identify epididymal cell types that help facilitate acute viral shedding. These studies will be crucial in identifying factors that dictate viral shedding in semen and will increase our understanding of how viruses are sexually transmitted.

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DIFFERENTIAL CUTANEOUS IMMUNE RESPONSES AND VIRAL DYNAMICS OF HISTORICAL AND NOVEL REASSORTANT OROPOUCHE VIRUS STRAINS IN HUMAN SKIN.

Drake Guo, Anthony Paivarinta, Aleena Alex, Isabelle Kahng, Cynthia M. McMillen, **Priscila MS Castanha**

University of Pittsburgh, Pittsburgh, PA, United States

Oropouche virus (OROV) is an emerging *Orthobunyavirus* causing significant outbreaks across Central/South America and the Caribbean. A novel reassortant strain is linked to expanded transmission, increased clinical severity, and reports of fatal and congenital cases. While historical OROV strains are well-studied, the pathogenesis and host-virus interactions of the novel circulating strain remain poorly defined. We employed a well-established human skin model to compare viral dynamics and early cutaneous immune responses between the historical strain (BeAn19991) and the novel circulating strain (240023). Explants were inoculated with 6 logs of OROV and cultured for 72 hours. Viral infection and skin-resident dendritic cell (DC) dynamics were evaluated via confocal microscopy and quantitative image analysis for OROV nucleoprotein (NP), CD1c (dermal DCs), and CD207 (Langerhans cells; LC). Viral RNA in tissues and migrated cells was quantified by RT-qPCR. Both strains readily infected the epidermal and dermal layers, with significantly higher viral replication observed in the dermis. While overall infection levels in these layers were comparable between strains, the historical BeAn19991 strain maintained significantly higher viral RNA levels within the infected tissue. Both strains induced significant recruitment of dermal DCs and LCs to the inoculation site compared to mock controls and demonstrated a similar capacity to infect these cell types. Notably, the novel 240023 strain induced significantly higher cellular migration out of the skin compared to the BeAn19991 strain. Migrated cells from both groups were positive for viral RNA, suggesting infection during egress, though viral RNA loads in these cells did not differ significantly between strains. These findings demonstrate that despite lower local tissue replication, the novel OROV strain induces greater cellular migration, suggesting an enhanced or altered early immune response. This strain-specific modulation of the initial cutaneous interface may contribute to the increased clinical severity and rapid dissemination observed in recent outbreaks.

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DEVELOPMENT OF A CONDITIONAL REPLICATION SYSTEM USING A LASSA VIRUS GLYCOPROTEIN COMPLEX-ENCODING RETROVIRAL VECTOR FOR ISOLATING RESISTANT VARIANTS TO INHIBITORS IN BSL-2

Hans Bakatumana Manya

Nagasaki University, Nagasaki City, Japan

Lassa virus (LASV), classified as a high-risk infectious disease or a Category A pathogen, demands strict biosafety level 4 (BSL-4) containment, limiting research due to few BSL-4 facilities. Consequently, replication-defective pseudotyped retroviral vectors are often used as safe alternatives for neutralizing activity assays of drugs and antibodies in BSL-2. Here, we established a novel retroviral vector system encoding the LASV glycoprotein complex (GPC) that replicates only in cells expressing murine leukemia virus (MLV) Gag-Pol proteins under BSL-2 conditions. Using this system, we isolated LASV GPC variants resistant to an anti-LASV compound, lamellarin α 20-sulfate, or a neutralizing antibody from a Lassa fever survivor. Resistance in lamellarin α 20-sulfate variants involved K125E and H13R amino acid substitutions, which cooperatively conferred resistance. The K125E enhanced infectivity and simultaneously conferred a lethal effect on cells in the conditional replication system, whereas the H13R mitigated this effect, thereby enabling stable expression of LASV GPC in cells. In neutralizing antibody-resistant variants, the I403T substitution conferred resistance by impairing antibody binding. This study provides a valuable BSL-2-based platform for isolating LASV GPC variants resistant to inhibitors.

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BARRIERS TO ENROLLMENT IN DENGUE FEVER ANTIVIRAL TRIALS: TURNING OBSTACLES INTO OPPORTUNITIES IN A PHASE 2A STUDY

Ruobing Li[1], Philipp Bless[2], Suraj Krishnamurthi[3], Diego Albrecht[2], Ana Blotta Baptista[4], Mitali Sant[5], Ana Paula Basso Rossi[4], Valerie TH Tan[5], Daniel Rooks[3], Renee Bergeron[6], Feng Gu[7], Linda Armstrong[6], Megan Barton[3], **Andreas Tietz**[2]

[1]Novartis Biomedical Research, Translational Medicine, Beijing, China, [2]Novartis Biomedical Research, Translational Medicine, Basel, Switzerland, [3]Novartis Biomedical Research, Translational Medicine, Cambridge, MA, United States, [4]Novartis Development, Global Clinical Operations, Sao Paulo, Brazil, [5]Novartis Development, Global Clinical Operations, Singapore, Singapore, [6]Novartis Biomedical Research, Translational Medicine, East Hanover, NJ, United States, [7]Novartis Biomedical Research, Global Health, Emeryville, CA, United States

Dengue infection is a growing global health problem, with an unmet medical need for effective and safe treatments. We report on the barriers and enhancers that impact recruitment in a Phase 2a multi-center, randomized, controlled study of EYU688, an oral dengue NS4B inhibitor, in adult patients with dengue fever (NCT06006559). The study is conducted in Brazil, Colombia, Mexico, India, Malaysia, Singapore and Vietnam. The main barriers to enrollment were: 1) Lower caseload in the 2025 dengue season compared to 2024 2) Challenges to conduct the full screening process within 48 hours after symptom onset 3) Baseline laboratory values indicate advanced disease in some cases, e.g. hematocrit, platelets and viral load 4) Limited sensitivity of the NS1 antigen rapid diagnostic test (RDT) as a screening tool. Results from more sensitive tests, like NS1 antigen ELISA and PCR are often not timely available 5) Protocol-mandated hospitalization for up to 5 days limits patients' willingness to participate. Home nursing as an alternative is not accepted in some countries 6) Regulatory and operational hurdles impede responsive opening of sites during outbreaks. The following mitigation measures were prioritized after a root cause analysis by a multidisciplinary team: 1) Enhanced accountability, focus and engagement between trial leadership and local project management 2) Community-centered recruitment strategies, e.g. patient referral through satellite clinics and public health partnerships, and screening of household contacts 3) Positive NS1 antigen ELISA was added to the inclusion criteria 4) Logistic and legal hurdles to home nursing were addressed 5) Intensified engagement with sites and investigators, fostering trust-building and best-practice sharing 6) Feasibility assessments for new sites take the above-mentioned challenges into account. The qualitative and quantitative impact of these measures on recruitment metrics will be reported at the congress. Insights into the success of mitigation measures can inform future antiviral treatment studies and help to optimize the roadmap for finding effective and safe treatments.

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CO-CULTURE SPHEROID MODELS TO STUDY DRUG-INDUCED LIVER INJURY IN DENGUE

Katherine Chan, Jasmin Broadey, Colin Osborne, Manjunatha Ujjini, Richard Eastman

Novartis Institutes for Biomed.Research, Emeryville, CA, United States

Dengue virus (DENV) infection is a growing global public health concern affecting an estimated 100-400 million people annually. Liver damage in dengue infection is common and ranges from mild AST, ALT enzyme elevation to fatal acute liver failure, and is multifactorial, involving direct viral cytopathic effects, cytokine storm, immune cell infiltration, etc. In preclinical testing, in vitro infection models and standard two-dimensional hepatocyte assays lack the physiological complexity required to accurately capture hepatocyte responses and as such, in clinical studies of novel antivirals, distinguishing viral driven hepatocellular damage from drug induced liver injury (DILI) can be a challenge. To address this limitation, we have developed a multicellular liver spheroid model infected with DENV using human primary hepatocytes and Kupffer cells, to quantify both dengue virus infection and compound induced hepatotoxicity, providing a more

accurate representation of human liver microenvironments during DENV infection. Using a luciferase-reporter DENV2 virus and semi-automated workflows, we optimized infection conditions, monitored viral kinetics, and assessed spheroid viability throughout the infection period. Exposure to DENV led to a time-dependent increase in hepatocellular injury biomarkers, such as ATP, LDH, AST, ALT, and albumin. When treated with known hepatotoxic compounds, cell damage was further exacerbated, highlighting the value of the co-culture spheroid model in detecting both viral cytopathic effects and compound-induced toxicity. The infected liver spheroid model provides an opportunity for a human relevant in vitro platform for evaluating hepatotoxicity during antiviral development. Enabling early compound triage, supporting informed decision making in medicinal chemistry, and guiding in vivo testing strategy and prioritization.

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PROXIMITY-BASED MAPPING OF THE NS3 PROTEIN INTERACTOME: COMPARING DUAL-HOST AND INSECT-SPECIFIC ORTHOFLAVIVIRUSES

Tyler R. Simpkins, Joshua Beck, Bradley J. Blitvich
Iowa State University, Ames, IA, United States

Orthoflaviviruses have diverse host specificities, with some capable of replicating in both vertebrate and mosquito hosts while others are restricted to a single host type. Although these viruses share similar genomic organizations, the molecular mechanisms that allow some viruses to infect multiple hosts remain poorly understood. This project seeks to identify vertebrate host proteins that interact with the viral nonstructural protein NS3, which is essential for the formation of the viral replication complex and suppression of the host immune response. To accomplish this, we employed TurboID biotin-based proximity labeling to identify host proteins that interact with NS3 from representative dual-host, insect-specific, and vertebrate-specific flaviviruses in both mosquito and mammalian cell lines. Labeled host proteins were isolated, identified using LC MS/MS, and analyzed using proteomic approaches to identify conserved and unique host-specific interactions. By defining these interaction networks, this work aims to provide insight into how different flaviviruses interact with host proteins and identify protein interactions essential for multi-host infection and those specific to single-host infection. Additionally, by identifying host proteins that are involved in viral replication and immune evasion, we can better understand the determinants of flavivirus host range restriction.

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SPATIO-TEMPORAL TRENDS OF ANTIMALARIAL DRUG RESISTANCE MARKERS IN SELECTED REGIONS OF MAINLAND TANZANIA FROM JANUARY 2021 TO AUGUST 2023

Laurencia Saidy Tangawizi[1], Dativa Pereus[2], Salehe S. Mandai[1], Rule Budodo[1], Hussein J. Semboja[1], Angelina J. Kisambale[1], Happyness A. Mwailunga[1], Baraka W. Mbuba[3], Gideoni I. Maulidi[1], Ninja S. Bisaga[4], Rashid A. Madebe[4], Catherine Bakari[1], Gervas Chacha[4], Doris David Mbata[4], Daniel P. Challe[5], Daniel A. Petro[6], Solomon Mickson Mwakasungula[1], Daniel Baraka Mbwambo[7], Sijenunu Aaron[7], Abdallah Lusasi[7], Samwel Lazaro[7], Stella Kajange[8], Celine I. Mandara[4], Misago D. Seth[4], Deus S. Ishengoma[1]
[1]Ifakara Health Institute, Dar Es Salaam, United Republic of Tanzania, [2]Muhimbili University of Health and Allied Sciences, Dar Es Salaam, United Republic of Tanzania, [3]Nelson Mandela African Institution of Science and Technology, Arusha, United Republic of Tanzania, [4]National Institute for Medical Research, Dar Es Salaam, United Republic of Tanzania, [5]National Institute for Medical Research, Tanga Research Centre, , Tanzania, Tanga, United Republic of Tanzania, [6]University of Dar es Salaam, Dar Es Salaam, United Republic of Tanzania, [7]National Malaria Control Programme, Dodoma, United Republic of Tanzania, [8]Prime Minister's Office, Regional Administration and Local Government, Dodoma, United Republic of Tanzania

Emergence and spread of antimalarial drug resistance threaten malaria control and elimination efforts. In this study, data from the Molecular Surveillance of Malaria in Tanzania project (2021-2023) were used to assess spatio-temporal trends in molecular markers associated with resistance to artemisinin, partner drugs, and sulphadoxine-pyrimethamine (SP). Dried blood spot samples were collected from 13 regions with varying transmission intensities, and sequenced on Illumina MiSeq platform. The *pfk13* R561H mutation showed regional variation, with higher prevalence in Kagera (5.89%) and Kigoma (3.36%) in 2023, and was low in Manyara (0.6%), Tabora (0.5%), and Njombe (0.4%) in 2021. In Kagera, most districts reported the mutation, with the highest prevalence in Karagwe (23.81%) in 2023. No temporal increase was observed, indicating stable prevalence. A675V increased (0.15% in 2021 to 0.68% in 2023); R622I in Njombe (0.3%) in 2021, in Dodoma (1.28%) and Mtwara (0.66%) in 2022, and observed only in Ruvuma (4.9%) in 2023. The *pfmdr1* Y184F allele ranged from 38–62% with regional variation, but no temporal trend. D1246Y (~2–5%) and N86Y (<2%) remained low. There was minimal spatial variation, with slightly higher D1246Y prevalence in Kagera (1.87%), suggesting sustained ACT partner-drug pressure. SP resistance markers, *pfdhfr* (N51I, S108N, C59R >90%) and *pfdhps* (A437G, K540E >90%) mutations remained high and near fixation. In contrast, *pfdhps* A581G showed marked spatial variation, from ~2% in Tabora to 53.78% in Tanga, and increased from 12-18% in 2021 to 25-35% in 2023. The *pfdhfr* I164L declined (~8% to ~2%) from ~8% in 2021 to ~2% in 2023, but remained higher in Kagera (17.03%) in 2023. *Pfcrt* mutations stayed low (<10%), with no variations. These results show that SP resistance is widespread with emerging higher-level resistance. Mutations linked to artemisinin partial resistance are rare but more common in Kagera and are now spreading to other regions. These results support region-specific molecular surveillance to guide control strategies and policy decisions, particularly for emerging drugs such as ganaplacide-lumefantrine.

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UNRESOLVED CHALLENGES IN THE VALIDATION OF GCN5 AS AN ANTIMALARIAL DRUG TARGET

Francisco-Javier Gamo[1], Pedro Martin-Acosta[1], Abbas H.K. Al Temimi[1], Luma Godoy Magalhaes[2], Juan Salamanca-Viloria[1], Beatriz Baragana[3], Sonia Moliner-Cubel[1]
[1]GlaxoSmithKline, Tres Cantos Madrid, Spain, [2]Drug Discovery Unit, Division of Biological Chemistry and Drug Discovery, Faculty of Life Sciences, University of Dundee, Dundee, United Kingdom, [3]1Drug Discovery Unit, Division of Biological Chemistry and Drug Discovery, Faculty of Life Sciences, University of Dundee, Dundee, United Kingdom

Artemisinin-based combination therapies (ACTs) have reduced malaria cases and deaths. However, increasing resistance to both artemisinin and its partner drugs is limiting their effectiveness and increasing the risk of failed treatments and malaria resurgence. Therefore, discovering and confirming new drug targets is vital to overcome resistance and maintain progress towards eradication and public health protection. *Plasmodium falciparum* GCN5 (PfGCN5) is a promising preclinical antimalarial target, with validation progressing in recent years through functional characterisation, genetic studies and initial chemical tractability. PfGCN5 is a histone acetyltransferase in a divergent SAGA-like complex, acting as a global epigenetic regulator by controlling transcription of genes linked to metabolism, development and drug response, making it an appealing regulatory target. Genetic studies suggest PfGCN5 as essential: conditional knockdown or disruption of key domains causes reduced growth, replication defects, impaired blood-stage survival, and reduced mosquito transmission, producing major transcriptional and developmental disturbances. Chemical validation is however nascent. Small-molecule bromodomain inhibitors can phenocopy genetic disruption and pharmacological inhibition has sensitised resistant parasites to artemisinin, but not fully validated PfGCN5 inhibitors exist and selectivity versus human homologues remains unresolved. Despite meeting many

desirable target criteria—essentiality for survival and transmission, central regulatory function, links to resistance pathways, enzymatic/druggable domains, and evidence that partial inhibition could be effective—PfGCN5 still faces several risks, including selectivity challenges, a lack of optimised inhibitors, limited in vivo validation, and unclear vulnerability thresholds. In this work we will present additional studies that address the need of further chemical validation.

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MALARIA BREAKTHROUGHS DURING SEASONAL MALARIA CHEMOPREVENTION IN NORTHERN KENYA: IMPLEMENTATION GAPS OR EMERGING RESISTANCE?

Diana Menya[1], Beatrice Rotich[2], Emmah Kimachas[2], David Ekai[3], Edwin Mbugua[4], Joseph Kipkoech[2], George Ambani[2], Lucy Abel[2], Dancan Wakoli[2], Caroline Ngina[2], Rebecca Lokwang[2], Wendy O'Meara[5]

[1]Moi University School of Public Health, Eldoret, Kenya, [2]Academic Model Providing Access to Health Care (AMPATH), Eldoret, Kenya, [3]Department of Health Services and Sanitation, Turkana County, Turkana, Kenya, [4]Catholic Relief Services (CRS), Kenya Program, Nairobi, Kenya, [5]Duke Global Health Institute, Duke University, Durham, NC, United States

In 2025, Seasonal Malaria Chemoprevention (SMC) utilizing Sulfadoxine-Pyrimethamine and Amodiaquine (SPAQ) was implemented across Turkana Central, Kenya. While SMC is known to significantly reduce the regional malaria burden, the emergence of "breakthrough" infections—malaria cases in children reportedly protected by the regimen—poses a threat to program efficacy. We utilized a "Sentinel Event" approach to identify specific gaps in protection and differentiate between implementation failures and potential biological resistance. Following the second SMC cycle in July 2025, surveillance was established at six public health facilities, ranging from Kanamkemer Sub-County Hospital to remote dispensaries like Kapua. A sentinel event was defined as an SMC-eligible child (3-59 months) presenting with a positive malaria diagnosis via RDT or microscopy. Detailed data were collected using KOBOCollect and Dried Blood Spot (DBS) samples were collected for real-time PCR confirmation and subsequent analysis of genetic markers of resistance to SP and AQ. Between June and October 2025, 705 children were enrolled. Although 89.6% had received at least one SMC cycle, 30% of all malaria-positive children fell ill during their window of peak protection (within 28 days of a confirmed, adherent dose). Notably, 66% of cases diagnosed by facility microscopy were PCR-negative, highlighting significant over-diagnosis. Among the true positives, implementation gaps were evident: 37.7% missed cycles due to travel, and 16.6% due to distributors not visiting. However, for the 30% of children who were fully adherent and up-to-date, clinical breakthroughs suggest either drug malabsorption linked to high regional malnutrition or emerging parasite resistance. While sentinel surveillance successfully identified real-time implementation gaps for immediate correction, the high breakthrough rate remains a concern. The results of the ongoing molecular analysis of DBS samples to be presented at the conference will be vital for determining if SPAQ remains an efficacious tool or if a shift in chemoprevention strategy is required.

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IDENTIFYING IMPLEMENTATION GAPS THROUGH SENTINEL EVENT SURVEILLANCE TO IMPROVE COVERAGE OF SEASONAL MALARIA CHEMOPREVENTION IN TURKANA, KENYA

Joseph Kipkoech Kirui[1], Diana Menya[1], Beatrice Chepkemoi Rotich[2], Emmah Chepkwemoi Kimachas[3], George Angolio Ambani[3], Lucy Achieng Abel[3], Dancan Wakoli[3], Caroline Ngina[3], Rebecca Lokwang[2], Elizabeth Ayoma[4], Victor Osiare[4], Wendy P. O'Meara[5]

[1]Moi University, Eldoret, Kenya, [2]Duke Global Inc, Nairobi, Kenya, [3]Academic Model Providing Access to Health Care (AMPATH), Eldoret, Kenya, [4]Catholic Relief Services, Turkana, Kenya, [5]Duke Global Health Institute, Duke University, Durham, NC, United States

Following a successful 2024 pilot that reduced malaria cases by over 70% in Turkana County, Kenya, the 2025 Seasonal Malaria Chemoprevention (SMC) campaign marked the second round of implementation and introduced a transition to the Electronic Community Health Information System (eCHIS). This evaluation used sentinel event surveillance to better understand where and why children were being missed, and how SMC delivery could be improved in real time. We conducted two intercycle assessments (ICAs) in June and July 2025, alongside facility-based sentinel event surveillance from July to October among children aged 3-59 months. In the ICAs, 260 households were purposively selected to assess SMC coverage, adherence, and the use of digital tools. Sentinel events defined as confirmed malaria cases among children eligible for SMC were identified across six health facilities. For each case, we conducted caregiver interviews and collected dried blood spots (DBS) to understand whether infections occurred due to missed SMC, incomplete dosing, or breakthrough despite treatment. SMC coverage improved from 55% in the first cycle to 66.7% in the second after early adjustments to the program. However, important gaps remained. Most missed doses occurred because Community Health Promoters (CHPs) did not reach households (58.3%) or because caregivers were away (22.2%), highlighting ongoing challenges with mobility and access. The introduction of eCHIS initially slowed delivery due to offline functionality issues, but use of the system improved from 59% to 87.5% by the second cycle. Sentinel surveillance identified 705 malaria cases, with 90% of children having received at least one SMC cycle, pointing to gaps in completing all cycles or maintaining protection over time. This findings demonstracte that by linking sentinel surveillance with rapid feedback, this approach helped identify practical solutions such as expanding outreach strategies and strengthening community engagement to improve coverage. We learnt that real-time monitoring can play a critical role in reaching missed children and strengthening SMC delivery in complex settings.

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EVALUATING ARTESUNATE-AMODIAQUINE (ASAQ) DEPLOYMENT WITH SEASONAL MALARIA CHEMO-PREVENTION: A SIMULATION STUDY OF RESISTANCE AND TREATMENT OUTCOMES

Sarit Adhikari[1], Kien Trung Tran[1], A. Fabrice SOME[2], Issiaka Soulama[3], Noufou Gansore[4], Carter C. Farinha[1], Aristide Nacoulma[5], Robert J. Zupko[6], Tran Dang Nguyen[7], Sidzabda Christian Bernard KOMPAORE[5], Frederic Wendlamita Nikiema[8], Farouk O. Sana[9], Maciej Boni[1]

[1]Temple University, Philadelphia, PA, United States, [2]IRSS-DRO, Hauts-Bassins, Burkina Faso, [3]Institut de Recherche en Sciences de la Santé (IRSS), Ouagadougou, Burkina Faso, [4]SP-PALU, Ouagadougou, Burkina Faso, [5]Ouagadougou, Burkina Faso, [6]Pennsylvania State University, State College, PA, United States, [7]Center for Computational Epidemiology and Infectious Diseases, H? Chí Minh, SG, Vietnam, [8]Houet, Burkina Faso, [9]Clinton Health Access Initiative, Ouagadougou, Burkina Faso

Seasonal Malaria Chemoprevention (SMC) is a highly effective preventive measure where the children at risk (3-59 months) are given antimalarials at monthly intervals during the high transmission season. We model and

integrate the SMC process in an individual-based simulation model of malaria transmission to study the combined impact of routine treatment and SMC on malaria-endemic countries like Burkina Faso. We use the WHO-recommended treatment regimen of 1 day of Sulfadoxine-Pyrimethamine and 3 days of Amodiaquine (SPAQ), along with coverage data provided by the national malaria control program of Burkina Faso. We aim to study the potential risk of deployment of artesunate-amodiaquine (ASAQ), currently the highest efficacy ACT in many African countries, along with SMC using SPAQ. We first calibrate the model so that the simulated efficacy aligns with observed SMC effectiveness in field studies. We then evaluate a range of intervention strategies over a ten-year period, both with and without SMC, to assess their relative impact. Switching to ASAQ under SMC is predicted to increase the 76T frequency by 87.24% relative to the status quo with SMC, while reducing monthly average treatment failures by 24.3% over the 10-year forecast period (2026-2035). The cycling and MFT strategies that incorporate ASAQ reduce treatment failures by 8.0% and 5.9%, respectively, when SMC is in place.

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FROM EVIDENCE TO POLICY: KENYA'S PROCESS OF DEVELOPING A NATIONAL MULTIPLE FIRST-LINE THERAPIES (MFT) STRATEGY FOR MALARIA

Ndinda Kusu[1], Sammy Mahugu[2], Edwin Onyango[2], Kibor Keitany[2], Morris Kiio[1], Paul Nyachae[1], George Opiyo Wadegu[1], Rima Shretta[3], Gladys Tetteh[3]

[1]Jhpiego, Nairobi, Kenya, [2]Ministry of Health, National Malaria Control Program, Nairobi, Kenya, [3]Jhpiego, Baltimore, MD, United States

Malaria remains a major public health burden in Kenya, with 5.5 million cases and 769 deaths reported in 2023. Emerging antimalarial resistance, including detection of kelch13 mutations in western Kenya, threatens recent gains. Although artemisinin-based combination therapies (ACTs) remain effective, proactive strategies are needed. Evidence shows that Multiple First-Line Therapies (MFT), using concurrent ACTs, can reduce drug pressure, prolong efficacy, and delay resistance. A pilot study in Homa Bay (2020-2022) treating 61,167 patients across 248 facilities demonstrated feasibility. From August 2025, the Ministry of Health-National Malaria Control Program (NMCP), with partners, undertook a rapid process to develop Kenya's first MFT implementation plan. Activities included co-creation workshops and multisectoral consultations involving government, researchers, civil society, donors, and partners. A mixed-methods evidence-to-policy approach used data from therapeutic efficacy studies, molecular surveillance, drug use, and routine surveillance. Modelling and stakeholder consultations informed ACT selection, deployment strategies, rotation timelines, and priority counties. Kenya adopted a phased two-year rotational approach combining geographic rollout with scheduled drug rotation. Forecasting and quantification were integrated into national supply planning, guided by feasibility, supply chain capacity, and health system readiness. Phase 1 of Rotation 1 will begin in October 2026 in four counties, with scale-up to all 47 counties by February 2028 and Rotation 2 will start in October 2028. Artesunate-pyronaridine and dihydroartemisinin-piperaquine are proposed for rotation 1 and 2, respectively. Training materials and reporting tools will be updated to support rollout. Integrating diverse evidence, strong stakeholder engagement, and piloting were critical. Robust surveillance, supply chains, and early planning are essential. Kenya's experience provides a practical model for translating evidence into policy for ACT diversification and can guide other malaria-endemic countries.

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LATE TREATMENT FAILURES OF ARTEMISININ-BASED COMBINATION THERAPIES IN IMPORTED *PLASMODIUM FALCIPARUM* MALARIA IN FRANCE (2013-2025): A CASE-CONTROL AND GENOMIC INVESTIGATION

Romain Coppée[1], Siwar Boufares[1], Anna Cosson[2], Noe Mathieuxx[3], Anthony Sleiman[3], Rizwana Zaffaroullah[1], Véronique Sarrasin[1], Nicolas Taudon[4], **Sandrine Houzé**[1]

[1]APHP Hôpital Bichat, Paris, France, [2]Université de Paris Cité, Paris, France, [3]Université de Rouen Normandie, Rouen, France, [4]IRBA, Brétigny sur Orge, France

Artemisinin-based combination therapies (ACTs) remain the cornerstone of *Plasmodium falciparum* malaria treatment. Late treatment failures (LTF) are sporadically reported in Africa, raising concerns about emerging drug resistance, suboptimal drug exposure, and host-related factors. Here, we investigated the incidence, epidemiological characteristics, and potential resistance determinants associated with LTF following ACTs in imported malaria cases in France. Between 2013 and 2025, we collected isolates from patients experiencing LTF after treatment with artemether-lumefantrine (AL), dihydroartemisinin-piperaquine (DHA-PPQ), or artesunate (AS). For each failure, two geographically and temporally matched controls with treatment success were included. Molecular analyses comprised genotyping of *pfkelch13*, *pfmdr1*, *pfcrt*, and *pfpm2/3* copy number variation. Clonal relatedness between day 0 (D0) and day of failure (Df) isolates was assessed using five microsatellite markers and whole-genome sequencing (WGS). A genome-wide association study (GWAS) comparing recurrent isolates with those from successfully treated patients was performed to identify novel markers associated with drug resistance. A total of 68 patients experienced LTFs during the study period. No known partner drug resistance mutations were detected, however novel mutations in *pfcrt* (M135L and C225W) were detected. No mutations were observed in the *pfkelch13* gene associated with artemisinin resistance. Microsatellite analysis and WGS confirmed recrudescence in most paired samples, with no emergence of *de novo* mutation emergence. GWAS identified one candidate non-synonymous mutation in the transporter-encoding *mrp1* gene (F1390I). LTFs among imported malaria cases in France remain uncommon and do not appear to be primarily driven by known resistance mechanisms. Recrudescence seems more likely related to inadequate drug exposure rather than within-host genetic adaptation during treatment. Continued genomic surveillance is essential to detect early signals of emerging partner-drug tolerance in parasites imported from Africa.

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EXPANDING THE ANTIMALARIAL ACTIVITY PROFILES OF DERIVATIVES OF PYRIDO[3,2-D]PYRIMIDIN-4(3H)-ONE AND QUINAZOLIN-4(3H)-ONE

Hagar Afriyie

University of Ghana (Drug Innovation group), Accra, Ghana

Phosphatidylinositol-4-kinase (PI4K) is an attractive target for a new generation of antimalarials because they are involved in various stages of the parasite lifecycle. Several hits containing the pyridopyrimidine moiety were identified from phenotypic screens against the malaria parasite. The pyridopyrimidines are heterocyclic aromatic compounds known for their medicinal properties. MMV900330 is one such compound in a chemical series that exhibits antimalarial activity in the phenotypic screen. Herein, we report the synthesis of thirty-nine novel small molecules inspired by phenotypic hit MMV900330. Two groups of compounds comprising quinazolin-4(3H)-one and pyrido[3,2-d]pyrimidin-4(3H)-one derivatives were prepared. The compounds were evaluated against a sensitive strain of the malaria parasite and exhibited low-micromolar activity *in vitro* against the 3D7 and NF54 strains. Generally, the quinazolin-4(3H)-one derivatives were found to be more active than their pyrido[3,2-d]pyrimidin-4(3H)-one variants. For example, SDD039 was threefold more potent than the resynthesized hit compound HA074 (IC50 = 0.16 μM), with an IC_{50} of 0.046 μM. However, the improved antimalarial activity of SDD039 did not translate into superior

ADME properties. It was less stable in human microsomes and rat hepatocytes than HA074, but had improved solubility. Compound HA069 returned a solubility of 135 μM, superior to 35 μM for the resynthesized hit compound.

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DISCORDANCE IN MOLECULAR CORRECTION OF RECURRENT MALARIA INFECTIONS IN EFFICACY TRIALS: LIMITATIONS OF WHO GENOTYPING ALGORITHMS AND INSIGHTS FROM AMPLICON SEQUENCING IN UGANDA

Thomas Katairo[1], Innocent Wiringilimaana[2], Bienvenu Nsengimaana[2], Francis D. Semakuba[2], Stephen Tukwasibwe[2], Jackie Nakasaanya[2], Victor Asua[2], Vester J. Muwanika[2], Alisen Ayitewala[3], Shakirah Babirye[2], Inna Gerlovina[4], Jerry Mulondo[2], Chris Ebong[2], Samuel Lubwama Nsobya[2], Adoke Yeka[2], BOSCO BEKIITA AGABA[5], Emmanuel Arinaitwe[2], Joaniter Nankabirwa[2], Isaac Ssewanyana[2], Martin James Donnelly[1], Philip J. Rosenthal[4], Bryan Greenhouse[4], Richard David Pearson[1], Melissa D. Conrad[6], Jessica Briggs[4]

[1]Liverpool School of Tropical Medicine (LSTM), Liverpool, United Kingdom, [2]Infectious Diseases Research Collaboration, Kampala, Uganda, [3]Central Public Health Laboratories, Kampala, Uganda, [4]University of California, San Francisco, CA, United States, [5]National Malaria Elimination Division, Kampala, Uganda, [6]John Hopkins University, Baltimore, MD, United States

The efficacy of artemether-lumefantrine, the first-line therapy for uncomplicated malaria in Uganda, is under threat, with high prevalences of PfK13 mutations associated with artemisinin partial resistance and decreased *ex vivo* susceptibility of *P. falciparum* to lumefantrine. Therapeutic efficacy studies (TESs) are the gold standard for evaluating antimalarial drug efficacy. However, in high-transmission settings, frequent reinfections after therapy complicate the identification of true treatment failures. Accurate determination of recrudescence requires the detection of matching parasite genotypes in day 0 (D0) and day of failure (DOF) samples. Currently, WHO recommends genotyping *msp1*, *msp2*, and a microsatellite, with 3 of 3 matches identifying recrudescence. However, this algorithm does not adjust for allele frequencies, complexity of infection or parasite relatedness. We compared the performances of microhaplotype-based genotyping, PfPHAST and a probabilistic-based analytical method (Aster) to the WHO algorithm using 109 paired D0 and DOF samples from a 2022-23 Ugandan TES. The 3/3 algorithm classified 80.7% (88/109) of failures as reinfections, 11.0% (12/109) as recrudescences, and 8.3% (9/109) as indeterminate. PfPHAST/Aster classified 78.9% (86/109) as reinfections, 12.8% (14/109) as recrudescences, and 8.3% (9/109) as indeterminate. The indeterminates identified by PfPHAST/Aster did not overlap with those identified by the WHO algorithm. Of the 91 cases with determinate calls by both algorithms, 15 (16.5%) were discordant, with only 3 (3.3%) cases called as recrudescent by both methods. Further study of re-extracted samples and mixed laboratory controls with known alleles is ongoing. In summary, most recurrent infections in the Ugandan TES were reinfections, recrudescences were seen in >10% of AL-treated patients with both algorithms, and there were important differences in results obtained with the two algorithms.

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RESHAPING *PLAMODIUM FALCIPARUM* PFDHFR/PFDHPS HAPLOTYPES WITH EXPANDING SEASONAL MALARIA CHEMOPREVENTION IN SENEGAL

Amy Gaye[1], Bassirou Ngom[1], Djiby Sow[1], Mamadou Alpha Diallo[1], Wesley Wong[2], Katelyn Vendrely Brenneman,[3], Aita Sene[1], Abdoulaye Tine[1], Yaye Die Ndiaye[1], Mariama Toure[1], Baba Dieye[1], Ibrahima Mbaye Ndiaye[1], Mamadou Samb Yade[1], Ndogaye Gadjaga[1], Cherif Diouf[1], River Mallick[3], Younousse Diedhiou[1], Jules Gomis[1], Elhadji Doucoure[4], Ibrahima DIALLO[4], Medoune Ndiop[4], Standeur Naby Kaly[4], Aliou Thiongane[4], Awa Bineta Deme[1], Bronwyn MacInnis[5], Dyann Wirth[2], Sarah K. Volkman[2], Daouda NDIAYE[1]

[1]Centre International de Recherche et de Formation en Génomique Appliquée et de la Surveillance Sanitaire, Dakar, Senegal, [2]Harvard T.H. Chan School of Public Health, Broad Institute, Boston, MA, United States, [3]Harvard T.H. Chan School of Public Health, Boston, MA, United States, [4]National Malaria Control Program (NMCP), Senegal, Dakar, Senegal, [5]Broad Institute, Cambridge, MA, United States

Antimalarial drug resistance threatens malaria control in Senegal as seasonal malaria chemoprevention (SMC) with sulfadoxine–pyrimethamine plus amodiaquine expands geographically and from 3 to 5 rounds per year in hightransmission areas. We hypothesized that intensified SMC exerts directional selection on *Plasmodium falciparum Pfdhfr* and *Pfdhps* haplotypes that confer resistance or fitness advantages. Using MAD4HatTeR amplicon sequencing, we characterized resistance haplotypes in 1,897 (2023) infections and 2,022 (2024) infections. Pairwise chisquare/Fisher's exact tests compared haplotype frequencies between years; siteadjusted binomial logistic regression quantified regional effects; and ecological Generalized Linear Models (GLMs) related SMC rounds and coverage to haplotype prevalence. The clearest, most consistent changes were in *Pfdhfr*/*Pfdhps*. From 2023 to 2024, *Pfdhfr* ICN declined in Kaolack (57.9%→32.3%, p=0.004) and Kolda (15.4%→4.3%, p=0.098) but rose in Diourbel (25.4%→40.1%, p=0.008). Each additional SMC round was associated with 79% lower odds of ICN (OR=0.21, 95%CI 0.12–0.34, p<0.001), while the triplemutant *Pfdhfr* IRN increased with SMC intensity (OR=1.77, p<0.001) and reached fixation in Tambacounda and Matam by 2024. For *Pfdhps*, VAGKGS fell sharply in Kaolack (74.2%→27.8%, p<0.001) and was negatively associated with SMC rounds (OR=0.15, p<0.001) and year (OR=0.50, p=0.015). An emerging *Pfdhps* haplotype, ISGEAA, appeared in five regions (up to 7.9% in 2024); although not yet significant in GLM (OR=3.53, p=0.257), its spread is worrisome. Siteadjusted models revealed marked regional heterogeneity, with Kedougou (5 SMC rounds) showing nearfixed IRN and low ICN/VAGKGS, with ICN positively associated with VAGKGS (OR = 1.37, 95% CI: 1.16–1.63, p < 0.001). These findings support the hypothesis that SMC deployment is reshaping *Pfdhfr*/*Pfdhps* haplotype structure and highlight the urgent need for functional characterization and sustained, nationally integrated molecular surveillance to safeguard firstline treatment and SPbased chemoprevention in Senegal.

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EX VIVO SUSCEPTIBILITIES OF *PLASMODIUM FALCIPARUM* TO STANDARD ANTIMALARIALS ACROSS UGANDA

Martin Okitwi[1], Stephen Orena[1], Oswald Byaruhanga[1], Gloria Cuu[1], Evans Muhanguzi[1], Jackson Rwatooro Asiimwe[1], Emmanuel Data[1], Patrick Angutoko[1], Solomon Opio[2], Ephraim Okello[1], Tonny Etwop[2], Yoweri Taremwa[1], Thomas Katairo[1], Stephen Tukwasibwe[1], Bersabeh Tafesse[3], Oriana Kreutzfeld[3], Jenny Legac[3], Samuel L. Nsobya[1], Melissa D. Conrad[4], Philip J. Rosenthal[3]

[1]Infectious Diseases Research Collaboration, Kampala, Uganda, [2]Dr. Ambrosoli Memorial Hospital Kalongo, Agago, Uganda, [3]University of California, San Francisco, San Francisco, CA, United States, [4]John Hopkins University, Baltimore, MD, United States

Artemisinin-based combination therapies (ACTs) are the current standard treatment for uncomplicated falciparum malaria, but their efficacy is threatened by artemisinin partial resistance (ART-R) and decreased susceptibility to ACT partner drugs. We evaluated ex-vivo susceptibilities to chloroquine (CQ), monodesethylamodiaquine (MDAQ), piperaquine (PQ), pyronaridine (Pyron), lumefantrine (LUM), mefloquine (MQ), dihydroartemisinin (DHA) and pyrimethamine (Pyrim) of *P. falciparum* isolates collected from January 2024 to February 2026 in eastern (E), northern (N) and southwestern (SW) Uganda, areas where ART-R associated PfK13 mutations (P441L, C469Y, C469F, R561H and A675V) have emerged. We collected 583 isolates (217, 296 and 90 from E, N and SW Uganda, respectively) from patients aged ≥0.5 years with confirmed symptomatic *P. falciparum* infections attending Kalongo Hospital and Patongo Health Centre (HC) IV in N Uganda, Busiu HC IV and Tororo District Hospital in E Uganda, and Karambi HC III, Kihihi HC IV, and Kamwezi HC III in SW Uganda. Ex-vivo drug susceptibilities were determined using a 72-hour growth inhibition (IC_{50}) assay (in Albumax-based complete culture medium) with SYBR Green detection. We found generally similar susceptibilities in E, N, and SW regions for all antimalarials tested, with only modest variations in median IC_{50} values (E vs N vs SW: CQ 15.5 vs 10.8 vs 20.5 nM, MDAQ 8.6 vs 6.6 vs 10.1 nM, PQ 6.3 vs 4.6 vs 10.1 nM, Pyron 1.3 vs 0.9 vs 1.5 nM, LUM 19.4 vs 17.0 vs 15.0 nM, MQ 23.4 vs 15.6 vs 23.7 nM, DHA 2.9 vs 4.2 vs 2.9 nM, and Pyrim 35,618 vs 30,450 vs 32,209 nM). Notably, modestly decreased LUM susceptibility, previously seen in N, and later E Uganda, appeared also to be present in SW Uganda. Susceptibilities to other ACT partner drugs remained excellent. Overall, these findings suggest comparable parasite susceptibilities to standard antimalarials across Uganda, with susceptibility to LUM somewhat decreased in recent years. Genomic analysis, to enable identification of genotype-phenotype associations, is ongoing.

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IMPLEMENTING MULTIPLE FIRST LINE THERAPIES TO ADDRESS ANTIMALARIAL DRUG RESISTANCE IN RWANDA: EARLY EXPERIENCE FROM FOUR STOP-AMDR-SUPPORTED DISTRICTS

Marcel Manariyo[1], Jean Modeste Harerimana[1], Noella Umulisa[1], Marie Rose Kayirangwa[1], Nadia Iriza[1], Jean Damascene Niyonzima[2], Anastase Muhashyi[2], Epaphrodite HABANABAKIZE[2], Aimable Mbituyumuremyi[2]

[1]STOP-AMDR, Jhpiego Rwanda, Kigali, Rwanda, [2]Malaria and Other Parasitic Diseases Division/Rwanda Biomedical Center, Kigali, Rwanda

Rwanda Malaria Surveillance data and Therapeutic Efficacy Studies (2018, 2021-2024) revealed emerging antimalarial drug resistance, including rising Pfkelch13 mutations A675V and R561H; and reduced artemether-lumefantrine efficacy to 88.1% below the 90% WHO threshold in study site. Malaria incidence rose sharply from 45 to 76 cases per 1,000 between 2023-2025, raising serious concerns about declining treatment effectiveness and emerging antimalarial drug resistance. In response, Rwanda adopted the WHOrecommended Multiple FirstLine Therapies (MFT) strategy. The STOPAMDR project supported the introduction of MFT in four districts: Gisagara, Kicukiro, Kayonza, and Musanze, since August 2025. Implementation followed by a coordinated healthsystems approach involving leadership engagement, hybrid and cascade training for healthcare providers, and targeted CHW training on blockspecific ACTs. The rollout was supported by updated guidelines, job aids, and social behavior change materials. Supply chain actions included withdrawing remaining AL stocks and distributing alternative ACTs complemented by ongoing supportive supervision and civil society engagement to reinforce adherence of community on MFT approach. A total of 658 healthcare providers and 5,264 CHWs were trained on malaria treatment, with an additional 2,092 CHWs sensitized on continued AL use. Within three months, 95% of health facilities and 87% of CHWs reported availability of appropriate malaria commodities. The treatment used in the first three months was diversified, with 62% AL, 25% DHAP, and 13% ASPY. Supportive supervision enabled redistribution of commodities, minimizing stockouts, and improving supply responsiveness. MFT implementation is feasible and rapidly operationalized within existing health systems. Rwanda's experience demonstrates how resistance surveillance can be translated into programmatic action through coordinated leadership, workforce capacity building, and adaptive supply systems, providing a scalable model in malaria high-burden settings.

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EVOLUTION OF *PLASMODIUM FALCIPARUM* ANTIMALARIAL DRUG RESISTANCE MARKERS IN UGANDA: 1998-2022

Mayland Treat[1], Brent Siegel[2], Victor Asua[3], Patrick Kumbategire Tumwebaze[4], STEPHEN TUKWASIBWE[5], Adoke Yeka[6], Grant Dorsey[7], Samuel Lubwama Nsobya[8], Moses R. Kamya[9], Philip Rosenthal[10], Melissa Conrad[11]

[1]University of California, San Francisco, San Francisco, CA, United States, [2]UCSF, HIV, Infectious Diseases, Global Health, Manhattan Beach, CA, United States, [3]IDRC, Kampala, Uganda, [4]Infectious Disease Research Collaboration, Kampala, Uganda, [5]Infectious Diseases Research Collaboration, KAMPALA, Uganda, [6]Infectious Dis Res Collaboration (IDRC), Kampala, Uganda, [7]Univ of California San Francisco, San Francisco, CA, United States, [8]Infectious Diseases Research Collaboration, Kampala, Uganda, [9]Makerere University, Kampala, Uganda, [10]Univ of California San Francisco, Mill Valley, CA, United States, [11]University of California, San Francisco, Baltimore, MD, United States

Resistance of *Plasmodium falciparum* to multiple drugs threatens the control of malaria in Africa. Standard therapy for uncomplicated malaria in Uganda was chloroquine (CQ) until 2000, CQ+sulfadoxine-pyrimethamine (SP) from 2000-2004, and artemether-lumefantrine since 2004, with slow implementation over the following years. To describe the evolution of antimalarial drug resistance in Uganda over the last ~25 years, we assessed prevalences of key resistance markers in samples collected from 7 different studies conducted from 1998 to 2022. Blood samples were collected from patients presenting with uncomplicated malaria in Kampala, central Uganda in 1998-99 and Tororo, eastern Uganda from 2004 to 2022. DNA was extracted from filter paper samples and sequenced using the molecular inversion probe platform with probes targeting 80 genes encoding potential resistance markers. The prevalence of markers associated with resistance to CQ and amodiaquine (*Pf*CRT K76T, *Pf*MDR1 N86Y and D1246Y) was high in early years of the study but declined to <10% by 2020. The prevalence of five SP resistance-mediating mutations in *P. falciparum* dihydrofolate reductase (*Pf*DHFR N51I, S108N, C59R) and dihydropteroate synthase (*Pf*DHPS A437G, K540E) increased to >90% by 2008, with subsequent increases in mutations mediating higher levels of resistance (*Pf*DHFR I164L to 25.0% in 2022; *Pf*DHPS A581G to 10.0% in 2022). The *Pf*K13 C469Y and A675V mutations, which are associated with artemisinin partial resistance and emerged in northern Uganda by 2016, were not seen in Tororo until 2022, but at that time had prevalences of 20.8% and 5.9%, respectively. Preliminary analysis of the remaining 75 potential resistance genes sequenced revealed some modest changes in polymorphism prevalences over time, but their relevance is uncertain; additional analysis is ongoing. Continued regular molecular surveillance is needed to monitor resistance trends and selective pressures driving resistance to help guide treatment and chemoprevention policy.

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INTEGRATING MULTIPLE FIRST-LINE THERAPIES INTO NATIONAL FORECASTING AND SUPPLY SYSTEMS: EARLY IMPLEMENTATION EVIDENCE AND SCALE-UP PLANNING FROM UGANDA

Steven Kikomeko[1], Thomson Ngabirano[1], Gerald Rukundo[2], Catherine Maiteki-Sebuguzi[2], Pamela Achii[2], Fredrick Makumbi[3], Gladys Tetteh[4], Naoko Doi[5], Lolade Oseni[5], Rima Shretta[5]

[1]Jhpiego, Kampala, Uganda, [2]Ministry of Health, Kampala, Uganda, [3]Makerere University School of Public Health, Kampala, Uganda, [4]Jhpiego, Washington, DC, United States, [5]Jhpiego, Baltimore, MD, United States

The WHO, in its 2022 strategy to respond to antimalarial drug resistance (AMR) in Africa, recommends deliberate deployment of Multiple First-Line Therapies (MFTs) to delay the emergence and spread of AMR. However, introducing multiple ACTs into centralized public-sector supply systems presents substantial operational challenges for forecasting, procurement, and distribution. Uganda is integrating MFT deployment within existing national health systems to support sustainable resistance mitigation. From 2025-2026, the Ministry of Health's National Malaria Elimination Division, in collaboration with National Medical Stores and partners under the STOP-AMDR project led by Jhpiego, incorporated MFT planning into national forecasting and procurement processes. ACT requirements were estimated using population data, age-stratified fever incidence, malaria positivity rates, projected trends in severe malaria, and routine consumption data from DHIS2. Forecasting incorporated impacts of vector control interventions, including ITNs and IRS, and accounted for treatment across public, PNFP, and community iCCM platforms, with buffer stock provisions to mitigate supply disruptions. National modelling and stakeholder consultations informed sub-national MFT deployment models aligned with facility levels and consumption patterns. One-year MFT commodities were secured for 90 facilities serving approximately 930,000 people in pilot districts. District allocation lists were integrated into routine national supply planning and scheduled for synchronized distribution through existing delivery systems. Findings demonstrate that MFT deployment can be operationalized within centralized national systems using existing governance and supply chain structures, improving treatment allocation, strengthening alignment between commodity supply and treatment needs, supporting more balanced ACT use, and reducing selective drug pressure while mitigating the risk of stockouts. This provides a practical and scalable model for integrating MFT into national systems and advancing AMR mitigation in high-burden settings.

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PREVALENCE OF ANTIMALARIAL DRUG RESISTANCE MARKERS AT FOUR REFUGEE SETTLEMENTS WITH VARIED MALARIA TRANSMISSION INTENSITY IN UGANDA

Stephen Tukwasibwe[1], Thomas Katairo[1], Francis D. Semakuba[1], Bienvenu Nsengimaana[1], Innocent Wiringilimaana[1], Gerald Mboowa[2], Monica Mbabazi[3], Brian A. Kagurusi[1], Victor Asua[4], Jackie Nakasaanya[1], Vester J. Muwanika[1], Isaiah Nabende[1], Alisen Ayitewala[5], Jerry Mulondo[1], Emmanuel Arinaitwe[1], BOSCO BEKIITA AGABA[6], Isaac Ssewanyana[7], Samuel Lubwama Nsobya[1], Moses R. Kamya[8], Bryan Greenhouse[9], Grant Dorsey[9], Jeffrey A. Bailey[10], Joaniter Nankabirwa[11], Jessica Briggs[12], Philip Rosenthal[13], Melissa Conrad[14]

[1]Infectious Diseases Research Collaboration, Kampala, Uganda, [2]Broad Institute, Cambridge, MA, United States, [3]Edinburgh, United Kingdom, [4]IDRC, Kampala, Uganda, [5]Central Public Health Laboratories (CPHL), Kampala, Uganda, Kampala, Uganda, [6]National Malaria Control Division-Uganda, Kampala, Uganda, [7]Infectious Diseases Research Collaboration (IDRC), Kampala, Uganda, [8]Makerere University, Kampala, Uganda, [9]Univ of California San Francisco, San Francisco, CA, United States, [10]Brown University, Providence, RI, United States, [11]Makerere Univ Kampala, Kampala, Uganda, [12]UCSF, San Francisco, CA, United States, [13]Univ of California San Francisco, Mill Valley, CA, United States, [14]8John Hopkins University Bloomberg School of Public Health, Baltimore, MD, United States

Antimalarial drug resistance is a significant and growing problem in Africa. Studying re-settled refugees is important, as this population is vulnerable to malaria due to substandard housing, a wide diversity of prior exposure to malaria parasites, and may import new malaria lineages, enabling resistance to spread. To evaluate this, we collected dried blood spots from 545 patients diagnosed with malaria at health facilities at Palorinya, Kiryandongo, Kyangwali and Nakivale refugee settlements in February-April 2025 and evaluated parasite drug resistance profiles using the PfPHAST amplicon panel, followed by sequencing on the Illumina platform. Palorinya is in Northern Uganda and is almost exclusively settled by S. Sudanese migrants, while Kiryandongo, in Western Uganda, hosts Sudanese and S. Sudanese. Kyangwali, in the west, and Nakivale, in the southwest, both host refugees from DRC. Two PfK13 mutations associated with artemisinin partial resistance were seen at modest prevalences at Palorinya (C469Y: 6.2%) and Kiryandongo (C469Y: 6.9%, A675V:7.1%) similar to what was observed in recent studies in newly arrived refugees from DRC, S. Sudan and Sudan and significantly lower than what was reported in nearby local host populations (Metu: C469Y: 23.3%, A675V: 9.1%; and Dima: C469Y: 37%, A675V: 10%). The prevalence of high-level antifolate resistance markers was high at settlements hosting refugees from DRC (*DHFR* I164L 25.9% and 13.7% and DHPS A581G 15.6% and 8.7% at Nakivale and Kyangwali, respectively) and low at Kiryandongo (*DHFR* I164L 4.9% and DHPS A581G 3.6%) and Palorinya (*DHFR* I164L 2.9% and DHPS A581G 1.8%) consistent with results from our previous studies. Chloroquine resistance marker, *CRT* K76T, uncommon elsewhere, was seen at 53% prevalence at Palorinya, presumably driven by refugees from and proximity to S. Sudan, where the first line antimalarial is artesunate-amodiaquine. Our findings show remarkable differences in prevalence of resistance markers across refugees from different countries and could inform policymakers on strategies to combat malaria and antimalarial drug resistance in refugee populations in Uganda.

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SYMPTOMATIC *PLASMODIUM FALCIPARUM* HRP2-BASED RAPID DIAGNOSTIC TEST POSITIVITY IN THE ABSENCE OF EVIDENT OR RECENT PARASITEMIA

Jillian L. Peters[1], Kayla Seggelke[2], Tamaki Kobayashi[2], Jay Sikalima[3], James L. Sichivula[3], Mike Chaponda[3], Jean-Bertin B. Kabuya[3], Yukari C. Manabe[1], William J. Moss[2], Matthew M. Ippolito[1]

[1]Johns Hopkins University School of Medicine, Baltimore, MD, United States, [2]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [3]Tropical Diseases Research Centre, Ndola, Zambia

Histidine-rich protein 2-based malaria rapid diagnostic tests (RDTs) can yield positive results when microscopy and PCR are negative. This is conventionally attributed to residual antigenemia following parasite clearance, but the clinical and biologic significance of isolated RDT positivity has not been systematically examined. We analyzed cross-sectional and longitudinal household surveillance visits (2012–2017) in holoendemic Nchelenge District, Zambia. Symptom history, PCR, RDT, microscopy, and hemoglobin were obtained. Visits were categorized as patent (PCR+/RDT+/microscopy+), subpatent (+/+/–), sub-RDT (+/–/–), RDT-only (–/+/–), or uninfected (–/–/–). Associations between diagnostic stratum and outcomes of symptom, anemia, or subsequent malaria were evaluated using mixed-effects logistic regression with a random intercept for individual ± household, adjusted for age and sex (and net use for subsequent malaria). Across 4,676 visits contributed by 3,215 participants, 42% were uninfected, 22% patent, 13% subpatent, 11% sub-RDT, and 11% RDT-only. RDT-only participants were younger and more often reported recent antimalarial use (34%). We subdivided RDT-only visits by evidence of recent malaria. Both subgroups had increased odds of any symptom (ORs 1.78, 1.82) and fever/chills (ORs 2.25, 2.43) vs. uninfected visits (P<0.005 for all comparisons). Anemia was increased only in the recent-malaria RDT-only subgroup (OR 2.03, P<0.001), whereas the no-recent-malaria RDT-only subgroup had

similar odds to the uninfected group (OR 1.07, P=0.72) despite exhibiting symptom profiles resembling patent infection. RDT-only status was not associated with subsequent PCR positivity, whereas subpatent and patent infection were (ORs 1.77, 1.93; P<0.05 for both). Isolated RDT positivity identified a symptomatic subgroup comparable to patent malaria despite negative PCR and microscopy. Findings were not fully explained by recent treated infection or increased short-term infection risk. The biologic basis and clinical implications of symptomatic isolated RDT positivity warrant further investigation.

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AWARENESS AND PERCEPTIONS OF CONCERNS ON GENETICALLY MODIFIED MOSQUITOES IN MALARIA CONTROL IN MALI THE NEED FOR SOCIALLY AND CULTURALLY LEGITIMATE GOVERNANCE

Samba Diarra[1], Sarah Hartley[2], Stelmach Aleksandra[3], Katie Ledingham[3], Abou Sogodogo[4], Seydou Doumbia[5]

[1]University of Sciences, Techniques and Technologies of Bamako; Department of Education and Research in Public Health and Specialties ;University Clinical Research Centre, Bamako, Mali, [2]Exeter University, Exeter, United Kingdom, [3]University of Exeter, Exeter, United Kingdom, [4]University Clinical Research Center, Bamako, Mali, [5]University Clinical Research Center, Faculty of Medicine, BAMAKO, Mali

6673 Background: Africa carries the highest global malaria burden, with over 90% of cases and deaths. The disease is primarily driven by the deadly *Plasmodium falciparum* parasite and transmitted by the highly efficient *Anopheles gambiae* mosquito. Sustained investment and innovation remain crucial to reducing malaria's impact on the continent. New ways of combating malaria include genetically modified mosquitoes (GMMs). However, like genetically modified organisms in general, the use of GMMs raises concerns and governance questions that need to be addressed. Methods: A qualitative study consisting of six focus group discussions (FGDs) was conducted in Koulikoro (rural) and Bamako (urban) in Mali. FGDs were conducted in the local language of Bamanankan, recorded, and transcribed for collaborative analysis. Data was coded manually, with themes and sub-themes extracted to address the study objectives. Results: GMMs are increasingly promoted as an innovative tool for vector control, yet public interpretations of this technology remain unevenly understood. Deep-rooted concerns about GMMs in different demographic and behavioral groups are revealed, such as anticipated effectiveness, risk profiles, institutional trust, perceived cost, and perceived fairness. This study highlights both convergences and divergences in the governance of GMMs in community perspectives on the introduction of GMMs across gender, geographic, and experiential lines. Interpretations of GMMs vary by gender, geography, and experience, yet communities share a common set of priorities. In conclusion. it is not enough for GMM to be scientifically effective for them to be accepted and used by beneficiary communities. They must also be socially and culturally legitimate and guided by governance that addresses both rational and emotional concerns. An information, education, and communication plan on GMMs needs to be developed with potentially beneficiary communities, without excluding any socio-professional strata.

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STRENGTHENING MALARIA DIAGNOSIS IN RESOURCE-LIMITED SETTINGS: HIGH ISO 15189 AWARENESS BUT CRITICAL SUPPLY CHAIN AND MENTORSHIP GAPS IN BONO EAST REGION, GHANA

Love Ankrah[1], Ernest Osei[2], Dennis Adu-Gyasi[1], David Dosoo[1]

[1]Kintampo Health Research Centre (GHS), Kintampo, Ghana, [2]Catholic University of Ghana, Sunyani-Fiapre, Ghana

Accurate malaria diagnosis is fundamental to case management and elimination efforts. Laboratory Quality Management Systems (LQMS) based on ISO 15189 guidelines ensure diagnostic reliability, yet implementation challenges persist in resource-constrained settings. Ghana's Bono East Region, a high-malaria-transmission zone, lacks data on the effect of LQMS on diagnostic quality. To assess factors affecting LQMS implementation for malaria diagnosis in health facilities in Bono East Region, Ghana, and identify priority areas for intervention, we conducted a cross-sectional study between June 2023 and April 2024 across 366 Bono East Region health facilities. A total of 210 laboratory personnel consented to participate in the study by using a standardized questionnaire to assess awareness of ISO 15189 guidelines, performance in key LQMS domains, and identify operational challenges. Data were analyzed using SPSS version 27. Awareness was categorized as high or low, with statistical significance set at p<0.05. Overall, 90.5% of laboratory personnel demonstrated a solid understanding of the components of ISO 15189. While laboratory organization and quality control scores were 98.5% and 96.0%, respectively, stock management (83.6%) and handling deviations from protocol (81.6%) were identified as areas for improvement. LQMS awareness was correlated with laboratory staff experience, with higher levels of awareness (p < 0.001) among individuals with over 11 years of experience. Lack of Giemsa stain (13.8%), Rapid Diagnostic Tests (RDTs) (13.6%), and malfunctioning microscopes (8.5%) were the limitations faced during laboratory activities. Laboratory professionals in the Bono East Region have a strong theoretical background in LQMS but face logistical challenges, including shortages of microscopy consumables and RDTs, which affect patient care. Strengthening the laboratory supply chain, providing mentorship, and conducting facility audits on LQMS and malaria diagnosis in the region may improve malaria case management and accelerate elimination efforts by identifying systemic gaps and strengthening quality assurance.

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IMPACT OF *PLASMODIUM FALCIPARUM* TRANSMISSION INTENSITY ON HIGH-SENSITIVITY RAPID DIAGNOSTIC TEST PERFORMANCE IN EARLY PREGNANCY

Jean Okitawutshu[1], Jeremiah Laktabai[2], Melissa Bauserman[3], Daja Denise Gaston[4], Fabian Omoding Esamai[5], Antoinette Tshefu Kitoto[6], Steve Myer Taylor[7]

[1]Kinshasa School of Public Health, Kinshasa, Democratic Republic of the Congo, [2]Moi University, Eldoret, Kenya, [3]University of North Carolina, Chapel Hill, NC, United States, [4]Durham, NC, United States, [5]Alliance for Maternal and Child Health, Busia, Kenya, [6]Kinshasa School of Public Health, Kinshasa, United States, [7]Duke University, Durham, NC, United States

Plasmodium falciparum infections during the 1st trimester of pregnancy are common in endemic areas and associated with poor pregnancy outcomes. Detection of parasites in the 1st trimester could be enhanced by using high-sensitivity rapid diagnostic tests (HS-RDTs). We lack an understanding of the performance of these tests for parasite detection in the 1st trimester. Additionally, we do not understand how parasite transmission intensity, by modifying the profiles of parasite densities among infected people, may impact test performance. We performed parasite detection by high-sensitivity rapid diagnostic test (HS-RDT) and by real-time PCR in women in the 1st trimester as a part of a clinical trial in DR Congo and Kenya. In rural clinics at each site, women were enrolled if they had ultrasound-confirmed singleton pregnancies ≤ 13 6/7 weeks gestation, were HIV-uninfected, and had not received recent antimalarials. Women were randomized to either usual care or to screening with the HS-RDT (Abbott NxTek Eliminate) followed by treatment with AL if positive. Participants were followed through delivery. From dried blood spots collected at the time of HS-RDT testing, we performed parasite detection and density estimation using a highly-sensitive qPCR assay, to allow for estimation of prevalence of parasites by each method, the density distribution, and the clinical sensitivity overall and by density of the HS-RDT in the 1st trimester. Overall, the parasite prevalence by HS-RDT was 52.6% (352/669) in DRC and 9.6% (39/405) in Kenya. In a prior preliminary analysis in DRC, we reported that the HS-RDT had moderate sensitivity (0.68) and specificity (0.77), relative to qPCR, which were both higher in primigravidae. Here, using data from all enrolled women, we will explore how local transmission intensity - expressed as qPCR parasite prevalence - modifies the observed parasite densities and

how this affects HS-RDT performance. These data will inform the suitability of high-sensitivity diagnostics for early pregnancy parasite detection in diverse endemic settings.

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ASSESSMENT OF ARTEMISININ-BASED COMBINATION THERAPY (ACT) AND MALARIA RAPID DIAGNOSTIC TESTS (RDT) UTILISATION IN SELECTED HOUSEHOLDS ACROSS SIX STATES IN NIGERIA IN 2024.

Segun Emmanuel Ibitoye[1], James Soujah Abu[1], Dawit Getachew[1], Olusola Oresanya[1], Kolawole Maxwell[1], Emmanuel Shekarau[2], Nnenna Ogbulafor[2]

[1]Malaria Consortium, Nigeria, Nigeria, [2]National Malaria Elimination Programme, Nigeria, Nigeria

The private sector provides approximately 55% of malaria treatment in Nigeria, however, evidence on household use of artemisinin-based combination therapies (ACTs) and rapid diagnostic tests (RDTs) across geopolitical zones remains limited. This study assessed the household malaria treatment and diagnostic patterns in six states. A cross-sectional household survey was conducted in six randomly selected states (Edo, Gombe, Kano, Kogi, Imo, and Osun), sampling 815 households across 24 Local Government Areas. Descriptive and bivariate analyses identified factors associated with ACT and RDT use. Overall, 65% of households reported increased malaria cases compared with one year before the survey, with rural households reporting higher rates (67%) than urban (62%). The northern region reported higher malaria case rates (82% in Kano) than the southern region (39% in Edo). Presumptive treatment was reported by 81% of households for both adults and children, primarily due to cost and ACT availability. Awareness of counterfeit ACTs was 59%. RDT use was significantly associated with education (p=0.025) and residence (p<0.001). ACT use was associated with residence in Kano (38.0% p<0.001), and was higher among males (68.3 vs 29.0%, p=0.015). Approximately 74% reported using ACT brands of Coartem, Lonart, and Amatem-Forte, with pharmacies and patent medicine vendors (PPMVs) as the primary source of care. Rural households opined that they patronise PPMVs due to their accessibility and lower costs. Only 75% of respondents completed their prescribed dosages, although concerns about counterfeit ACTs persisted. Approximately 76% of households reported out-of-pocket spending, of which 15% spent over NGN 20,000 annually on malaria treatment. Despite improved awareness of ACTs and RDT use, gender and regional disparities persist in malaria diagnosis and treatment in Nigeria. Subnationally tailored, multilevel interventions are needed to strengthen malaria case management, reduce counterfeit medicines and price volatility, particularly in rural areas where access and affordability remain critical barriers.

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HIGH RATE OF *PLASMODIUM OVALE CURTISI* MALARIA AMONG CHILDREN IN ANKILILOAKA, SOUTHWESTERN MADAGASCAR

Dina Ny Aina Liantsoa Randriamiarinjatovo[1], Seheno Razanatsiorimalala[1], Arsène Indriambelo[2], Laurence Randrianasolo[1], Milijaona Randrianarivelojosia[1]

[1]Institut Pasteur de Madagascar, Antananarivo, Madagascar, [2]Université de Toliara, Toliara, Madagascar

Malaria remains a major public health concern in Madagascar, with a marked increase in incidence since 2015. While *Plasmodium falciparum* predominates, data on less common species such as *P. ovale* remain scarce. Southwestern Madagascar, despite its predominantly subarid climate, remains prone to recurrent malaria epidemics, particularly in communities located in proximity to water bodies such as natural springs and irrigated rice fields. This study assessed the prevalence of *P. ovale curtisi* and *P. ovale wallikeri* among children under 15 years of age in three villages in Ankililoaka (southwestern Madagascar). Dried blood spot samples collected in 2023 (n = 373) were analyzed using nested PCR. Malaria infection was confirmed by PCR in 198 cases (53.1%, 95% CI: 47.9-58.2%). Among infected individuals, 36 cases of *P. ovale* were identified (18.2%, 95% CI: 13.2-24.4%), including 28 *P. ovale curtisi*, 7 *P. ovale wallikeri*, and one mixed infection. This study demonstrates the coexistence of *P. ovale curtisi* and *P. ovale wallikeri* in southwestern Madagascar. Interestingly, the proportion of *P. ovale* infections was higher in the three study sites (18.2%; 36/198 PCR-confirmed malaria cases) compared with that observed in our previous study conducted in eastern Madagascar (2.7%; 12/438 PCR-confirmed cases). *P. ovale curtisi* rate is high (77.8%; 29/36). Further large-scale studies are warranted to better characterize the distribution of *P. ovale* in the arid southern regions of Madagascar and to elucidate its epidemiological significance as a hypnozoite-forming parasite, thereby informing locally adapted malaria control strategies, particularly with respect to antimalarial use.

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WHAT INFORMATION DOES THE MALARIA RAPID DIAGNOSTIC TEST (MRDT) PROVIDE DURING A MALARIA OUTBREAK INVESTIGATION IN VANGAINDRANO, SOUTHEASTERN MADAGASCAR?

Seheno Razanatsiorimalala[1], Dina Ny Aina Liantsoa Randriamiarinjatovo[1], Yvette Razafimaharo[2], Aina Harimanana[1], Milijaona Randrianarivelojosia[1]

[1]Institut Pasteur de Madagascar, Antananarivo, Madagascar, [2]Programme Nationale de Lutte contre le Paludisme, Ministère de la santé publique, Madagascar, Antananarivo, Madagascar

Malaria transmission is perennial in the health district of Vangaindrano, located in the humid southeastern coastal region of Madagascar. Routine data from primary health centers indicated an unusual increase in malaria cases among outpatients beginning in September 2022. Following a request from the National Malaria Control Program, an outbreak investigation was conducted in February 2023. Active case detection was carried out in two geographically distinct remote areas. In selected households, all residents were screened using capillary blood obtained by finger prick. A malaria rapid diagnostic test (mRDT; Meriscreen, detecting pan-LDH and pfHRP2 antigens) was performed, and thick blood smears were prepared using 6 µL of blood for microscopy. Among 453 individuals screened, 182 tested positive by mRDT, yielding a positivity rate of 40.2% (95% CI: 35.7–44.9). Using microscopy as the reference standard, 63 (13.9%) results were false positives and 21 (4.6%) were false negatives. The sensitivity and specificity of the mRDT were 85% (95% CI: 78.2–90.0) and 79.9% (95% CI: 75.1–83.9), respectively. Agreement between mRDT and microscopy was moderate (Cohen's kappa = 0.6). The high mRDT positivity rate among community members confirmed the occurrence of a malaria outbreak, even though the investigation was conducted several months after the initial alert. The proportion of false-positive results, likely due to HRP2 antigen persistence, reflects recent transmission intensity. These findings highlight the operational value of mRDTs for rapid outbreak confirmation and decision-making at peripheral levels, without reliance on central laboratory support. In similar endemic settings, an mRDT positivity rate exceeding 30% among the first 100 individuals tested may serve as a pragmatic threshold to trigger mass drug administration and accelerate outbreak control

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QUALITY OF INPATIENT TEST-AND-TREAT MALARIA CASE-MANAGEMENT IN PUBLIC AND PRIVATE HOSPITALS IN KANO STATE, NIGERIA

Dawit Getachew[1], Nnenna Ogbulafor[2], Emmanuel Shekarau[2], Babangida Musa[3], Abdullahi Yusuf[3], Oladipo O Oladosu[4], Olusola Oresanya[1], Kolawole Maxwell[1], Dejan Zurovac[5]

[1]Malaria Consortium, Abuja, Nigeria, [2]National Malaria Elimination Programme, Abuja, Nigeria, [3]Kano State Malaria Elimination Programme, Kano, Nigeria, [4]Bowen University, Iwo, Nigeria, [5]Independent Consultant, Zagreb, Croatia

Compliance with evidence-based treatment guidelines supported by quality-assured parasitological diagnosis is central to effective malaria case

management in Nigeria. A cross-sectional assessment was conducted to evaluate compliance with test-and-treat malaria guidelines and routine microscopy performance in 18 public and private hospitals in Kano State, Nigeria. Data collection included hospital assessments, health-worker interviews, review of paediatric and medical ward admissions for August 2024, and rechecking of malaria slides archived over three months. Analyses included 18 hospitals, 72 health workers, 2,814 suspected malaria admissions, and 211 malaria slides. Nearly all hospitals (94.4%) provided parasitological diagnosis and stocked recommended antimalarials. Most health workers had received training on severe malaria (73.6%), but only 16.7% received supportive supervision. Composite test-and-treat performance was 39.3%, higher in children than adults (45.7% vs 26.5%) and in public vs private hospitals (39.8% vs 30.8%). Among suspected cases, 73.7% were tested on admission and 90.2% of severe malaria cases were treated with artesunate. Children were more often tested (79.8% vs 61.7%) and treated with artesunate (93.5% vs 80.1%). Patients in private hospitals were more often tested (84.3% vs 73.0%) but less often treated with artesunate (73.1% vs 91.2%). Only 30.0% of artesunate-treated patients were prescribed ACT—more commonly in adults (48.3% vs 23.0%) and in private hospitals (89.2% vs 26.9%). ACT use for admitted non-severe cases was rare (2.4%), while non-compliance with negative test results was high (75.8%). Routine microscopy showed 93.2% sensitivity, 42.5% specificity, 29.9% positive predictive value and 95.9% negative predictive value. Inpatient compliance with malaria test-and-treat guidelines varied across tasks, age groups and hospital sectors. Clinicians can trust negative slides but should interpret positive results cautiously. Strengthening malaria diagnostic quality assurance and continuous quality improvement is needed to improve inpatient malaria case management.

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IMPROVING QUALITY OF INPATIENT TEST AND TREAT MALARIA CASE MANAGEMENT USING JOINT CLINICAL AND LABORATORY STRATEGY IN KANO STATE, NIGERIA

Dawit Getachew[1], Nnenna Ogbulafor[2], Emmanuel Shekarau[2], Babangida Musa[3], Abdullahi Yusuf[3], Oladipo O Oladosu[4], Olusola Oresanya[1], Kolawole Maxwell[1], Dejan Zurovac[5]

[1]Malaria Consortium, Abuja, Nigeria, [2]National Malaria Elimination Programme, Abuja, Nigeria, [3]Kano State Malaria Elimination Programme, Kano, Nigeria, [4]Bowen University, Iwo, Nigeria, [5]Independent Consultant, Zagreb, Croatia

Quality of care and compliance with treatment guidelines supported by quality-assured parasitological diagnosis are central to inpatient malaria case management in Nigeria. However, inpatient quality-of-care data remain scarce and linkages between clinical and laboratory quality improvement interventions are often absent. We implemented and evaluated a joint clinical-laboratory strategy to improve inpatient test-and-treat malaria case management. The strategy combined annual hospital assessments with joint quarterly clinical-laboratory quality improvement interventions in 18 public and private hospitals in Kano State, Nigeria. The evaluation compared baseline and follow-up assessments after the first annual cycle. Data collection included hospital assessments, health-worker interviews, rechecking routine malaria slides by expert microscopists, and review of paediatric and medical ward admissions in August 2024 and August 2025. At baseline vs follow-up, analyses included 18 hospitals, 72 health workers, 2,743 vs 3,198 suspected malaria admissions, and 211 vs 270 rechecked malaria slides. Minimum test-and-treat readiness, defined as availability of malaria diagnostics, injectable artesunate, and ACTs, was observed in 94.4% of hospitals at baseline and 88.9% at follow-up. Composite test-and-treat compliance improved from 13.0% to 55.4% in public hospitals (11.9% to 55.8%) and private hospitals (29.7% to 51.1%), and among paediatric (12.2% to 64.2%) and adult inpatients (14.7% to 40.7%). Performance of individual tasks improved: malaria testing on admission (73.7% to 89.9%), artesunate treatment for severe malaria (90.2% to 91.2%), ACT follow-on treatment (29.8% to 85.6%), ACT treatment for non-severe malaria admissions (2.4% to 7.4%), and compliance with negative test results (24.2% to 52.8%). Completion of one annual cycle of the joint clinical-laboratory strategy substantially improved inpatient malaria case management across age groups and hospital sectors. Continued implementation with repeated practice assessments is needed to sustain and further improve performance.

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LIMITED AVAILABILITY AND USE OF MALARIA RAPID DIAGNOSTIC TESTS IN PRIVATE SECTOR OUTLETS: RESULTS FROM ACTWATCH LITE STUDIES IN BENIN, CAMEROON AND NIGERIA

Keith Esch[1], Katelyn Woolheater[1], Manfred Accrombessi[2], Erica Wang[1], Paul Bouanchaud[1]

[1]PSI, Washington, DC, United States, [2]PSI, Cotonou, Benin

The WHO recommends all people with suspected malaria receive a confirmatory test prior to treatment. In Benin, Cameroon and Nigeria, 44%-54% of children under five with fever for whom advice or treatment was sought did so in the private sector. However, private outlets often operate independently of formal government supervision and surveillance mechanisms, resulting in limited information on diagnostic access, quality, and use. ACTwatch Lite is a Gates Foundation-funded streamlined malaria market study methodology through which malaria diagnostic, treatment and provider data are collected at both formal and informal private sector outlets using representative, cross-sectional, probability sampling of study areas. Data from Benin, Cameroon (Centre and Littoral), and Nigeria (Abia, Kano and Lagos) were analyzed to assess indicators including availability, market composition, price and provider knowledge of malaria rapid diagnostic tests (RDTs). In Benin, 37.2 (95% CI 29%-45%) of private outlets stocked an RDT. In the formal private sector, clinics/hospitals stocked significantly more RDTs (60.3%; 95% CI 49%-71%) than pharmacies (1.2%; 95% CI 0.2%-6%). Only 1.2% (95% CI 0.2-8%) of informal outlets in Benin stocked an RDT. In Cameroon, RDT availability was significantly higher in the formal outlets of Centre (55.5%; 95% CI 46%-65%) and Littoral regions (33.4%; 95% CI 26%-42%) than informal outlets (7%; 95% CI 2%-15% and 16%; 95% CI 10%-25%), respectively. Across regions and outlet types, 59% had never performed an RDT on a client. Significantly more patent and proprietary medicine vendors in Kano, Nigeria, stocked an RDT (31.2%; 95% CI 27%-35%) than in Abia (0.1%; 95% CI 0%-0.3%) or Lagos (0.4%; 95% CI 0.2%-1%). Across states, price of testing was the same or higher than most treatments. Expanded access to affordable malaria diagnostics in the private sector is critical to the promotion of appropriate test-and-treat practices. ACTwatch Lite fills crucial evidence gaps in private sector malaria diagnosis practices to enable policy makers to target interventions that improve access and quality of care.

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IMPROVED DIAGNOSTICS IN ADVANCING UNIVERSAL HEALTH COVERAGE; PREVALENCE OF *PFHRP2* AND *PFHRP3* GENETIC VARIABILITY AND THE IMPACT ON MALARIA DIAGNOSIS IN KWALE, KENYA

Francis T. Kimani, William Chege, Maureen Otinga, Noah Machuki, Lewis Karani, Mary Ombati, Eva Nambati, Kelvin Thiong'o, Lynette Wangechi

Kenya Medical Research Institute, Nairobi, Kenya

Accurate *Plasmodium falciparum* malaria diagnosis remains key in reducing the impact of a disease that is putting 70% of the Kenyans at risk. Rapid diagnostic test (RDT) kits that rely on the detection of *P. falciparum* histidine-rich protein 2 (*Pfhrp-2*) offers the ability to accurately diagnose malaria while retaining diagnostic efficacy. Reports have shown the emergence of mutant parasites with deletions in the *these* gene(s) that may derail this. This study sought to investigate these deletions in Kenya. We hypothesized that areas of endemic malaria transmission in Kenya do not have any of these gene deletions. A cross-sectional study was conducted in Ten health facilities from Kwale County, a Coastal malaria endemic region. These sites were sampled to ensure geo-representation and capture the malaria distribution. Sample size was based on the WHO recommendation for *Pfhrp2* deletions while following the inclusion criteria outlined. Confirmed *pf*-positive samples

were analyzed for the presence of two genes, including their upstream and downstream flanking regions with appropriate controls of known deletions status. A total of 376 dry blood spot samples were collected from febrile patients across the 10 facilities. The analysis results had 19 (8.6%) of the 221 *Pf* positive samples showing a deletion of the *pfhrp2* gene and 61 (27.6%) for the *pfhrp3* gene. Cross-reactivity was observed in the study, with 10 (4.5%) samples showing deletion of *pfhrp2* but positive for *pfhrp3* and 52 (23.5%) samples positive for *pfhrp2* but negative for *pfhrp3*. Further 9 (4.1%) of samples showed *pfhrp2/pfhrp3* double deletions. One was RDT negative, microscopy positive with both deletions. The rest had positive RDT and/or microscopy results but were negative for *pfhrp2, pfhrp3*, or both. This is a concern on the reliability of RDTs targeting *Pfhrp-2*. This will provide the NMCP with data on the current burden of these deletions and add to the WHO's emergent pool of data on mutation-driven false negative results in *Pfhrp-2* based kits in tandem with ongoing antimalarial drug efficacy monitoring programs. Ultimately, this will guide the development of improved malaria RDT tools.

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USING SIMULATED MALARIA CASES TO CLOSE COMPETENCY ASSESSMENT GAPS IN LOW PATIENT VOLUME FACILITIES: EXPERIENCE FROM OUTREACH TRAINING AND SUPPORTIVE SUPERVISION (OTSS) IMPLEMENTATION IN EQUATORIAL GUINEA

Julie Buekens[1], Maria Consuelo Oki Eburi[2], Adrian May[2], Elizabeth Lucas Nyakarungu[2], IniAbasi Nglass[2], José Raso[3], Jordan M. Smith[1], Matilde Riloha Rivas[3], Wonder Philip Phiri[2], Carlos Alberto Guerra[4], Guillermo A. Garcia[1]

[1]MCD Global Health, Hallowell, ME, United States, [2]MCD Global Health, Malabo, Equatorial Guinea, [3]National Malaria Control Program, Malabo, Equatorial Guinea, [4]MCD Global Health, Quito, Ecuador

Outreach Training and Supportive Supervision (OTSS) relies on direct observation of clinical and diagnostic practice to assess health care worker competencies using a digital checklist organized by pre-determined categories. During certain OTSS visits, especially in low-patient health facilities, trained supervisors may not be able to observe all checklist categories, leading to incomplete competency assessments and gaps in quality assurance. In 2026, the Bioko Island Malaria Elimination Project (BIMEP) in collaboration with the Ministry of Health and the National Malaria Control Program (NMCP), expanded OTSS visits at the national level, thereby broadening the range of facilities visited, including hospitals, health centers, and health posts. Simulated cases were introduced into both clinical and laboratory checklists to mitigate potential gaps due to patient volume variability. During clinical supervisions, trained supervisors will use clinical vignettes representing uncomplicated malaria, severe malaria, and malaria in pregnancy when real cases are absent. Supervisors will simulate rapid diagnostic tests and microscopy scenarios when needed during laboratory supervisions. As with real cases, simulations will be followed by immediate feedback and on-site coaching, consistent with the OTSS approach. The number of simulated versus real cases will be compared across facilities and facility types, as well as within checklists to assess the number of supervision categories evaluated through simulations. It is anticipated that the use of simulated cases will be particularly useful in the smaller health facilities, enabling consistent evaluation of malaria case management skills regardless of case burden. Integrating simulated malaria cases into OTSS is a practical and replicable adaptation that strengthens competency assessment in facilities where patient volume cannot be controlled.

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PRIORITIZING COMPUTER LITERACY AS PART MALARIA CASE MANAGEMENT TRAINING OF TRAINERS

Julie Buekens[1], Maria Consuelo Oki Eburi[2], Adrian May[2], IniAbasi Nglass[2], José Raso[3], Jose Armando Riloha Dansu[2], Duncan McFarland[1], Matilde Riloha Rivas[3], Wonder Philip Phiri[2], Carlos Alberto Guerra[4], Guillermo A. Garcia[1]

[1]MCD Global Health, Hallowell, ME, United States, [2]MCD Global Health, Malabo, Equatorial Guinea, [3]National Malaria Control Program, Malabo, Equatorial Guinea, [4]MCD Global Health, Quito, Ecuador

Effective decentralization of malaria case management training depends on newly certified trainers being able to independently manage the practical tools essential to their work. In many public health training programs, particularly those designed to cascade technical knowledge through multiple levels of a health system, curricula assume a baseline level of digital competency that is rarely assessed before delivery. When that assumption goes unchecked, the cascade breaks down: trainers return to their districts equipped with technical knowledge but unable to independently access or adapt materials, prepare reports, or organize follow-on training sessions. The Bioko Island Malaria Elimination Project (BIMEP), in collaboration with the Ministry of Health and the National Malaria Control Program (NMCP) of Equatorial Guinea, identified this gap through direct observation and participant interaction across a series of case management Training-of-Trainers (TOT) sessions. Participants themselves confirmed what TOT facilitators observed: basic computer skills needed for this work were either absent or underdeveloped. Most participants spend the majority of their working hours in clinical or diagnostic practice with little or no routine use of computers. In response, the TOT curriculum was extended to include a dedicated day of foundational computer skills training covering word processing, presentation software, email, and basic document organization, delivered with support from MCD Global Health and BIMEP technical staff. Between August 2024 and August 2025, three adapted TOTs were held across Malabo, Baney, and Luba districts, reaching approximately 30 participants. The 2026 implementation cycle marks the first year in which these trainers will conduct district-level refresher sessions with decentralized support. Outcomes from this cycle will provide the first direct test of whether the curriculum adaptation translated into functional trainer independence. Designing TOT programs for true decentralization requires accounting for the full set of competencies trainers will need, including those often overlooked.

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ESTABLISHING THE FIRST EXTERNAL QUALITY ASSESSMENT (EQA) PROGRAM FOR MALARIA MICROSCOPY IN EQUATORIAL GUINEA: A PILOT STUDY IN THREE REFERRAL HOSPITALS

Elizabeth Nyakarungu[1], Maria Consuelo Oki Eburi[1], Jose Raso Bijeri[2], Adrian Eho May[1], Monserrat Kobe Elonga[2], Rosalia Nzang Bibang[2], Thomas Carl Stabler[3], Ilda Elobe Mometolo[2], Alejandro Lopelo Bolopa[2], Jose Maria Ntutumu[2], Teodora Mikumu Alogo[2], Cristina Nka Esokolete[2], Wonder Philip Phiri[1], IniAbasi Nglass[1], Julie Guadalupe Buekens[3], Duncan McFarland[3], Florentino Abaga Ndoho[2], Matilde Riloha Rivas[2], David Galick[1], Carlos Alberto Guerra[4], Claudia Daubenberger[5], Guillermo A. Garcia[3]

[1]MCD Global Health, Malabo, Equatorial Guinea, [2]National Malaria Control Program, Malabo, Equatorial Guinea, [3]MCD Global Health, Hallowell, ME, United States, [4]MCD Global Health, Quito, Ecuador, [5]Swiss Tropical and Public Hlth Inst, Basel, Switzerland

In 2024-2025, the Baney Reference Laboratory (BRL), the national malaria reference center in Equatorial Guinea (EG), participated in international External Quality Assessment (EQA) programs to evaluate diagnostic performance in malaria microscopy. Baney received 10 validated proficiency slide panels per survey from the National Institute for Communicable Diseases (NICD), South Africa. Results from six surveys (three per year) showed microscopy detection accuracies of 100% in 2024 and 95.5% in

2025 against an acceptable threshold of 80%, confirming strong detection accuracy. Quantitation scores averaged 80% but varied (2024: 50%, 95%, 80%; 2025: 88%, 96%, 71%), with two surveys rated *not acceptable*. These results indicated reliable parasite and species identification but inconsistent parasite density estimation. Building on these findings, the BRL, through the National Malaria Control Program (NMCP) and Bioko Island Malaria Elimination Project (BIMEP), will conduct EG's first national EQA pilot from May to September 2026 in three referral hospitals: Sampaka General, Malabo Regional, and Bata Regional. The pilot will use blind slide panels prepared from the National Malaria Slide Bank set at BRL and follow national EQA procedures modeled on EG's EQA guidelines, emphasizing manual slide rechecking by expert microscopists. Performance will be assessed for parasite detection, species identification, quantitation accuracy, staining quality, and interlaboratory concordance following WHO-AFRO standards. Preliminary data will be available in August 2026, providing the first national benchmark for microscopy quality assurance. Establishing this EQA program will strengthen malaria diagnosis and improve surveillance data quality, directly supporting case management and EG's progress toward malaria elimination.

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PLASMODIUM FALCIPARUM AND *P. VIVAX* DIAGNOSIS BY RAPID TEST DOES NOT DIFFER BETWEEN VENOUS AND CAPILLARY BLOOD

Cristian Koepfli[1], Kifle Negash[2], Birhanu Lulu[2], Yalemwork Ewnetu[3], Dawit Hawaria Logita[4]

[1]University of Notre Dame, Notre Dame, IN, United States, [2]Hawassa University, Hawassa, Ethiopia, [3]University of Gondar, Gondar, Ethiopia, [4]Hawassa university, Hawasa, Ethiopia

Rapid diagnostic tests (RDTs) are now the most widely used method for malaria diagnosis. At clinics, blood for RDT testing is collected either by finger prick (for capillary blood) or through phlebotomy (for venous blood). Because parasites often sequester in inner organs, the density of antigens detected by RDTs might differ between venous and capillary blood. We collected 1060 paired venous and capillary blood samples from febrile patients in Ethiopia and compared RDTs detecting *Plasmodium falciparum* by HRP2 and LDH (BIOCREDIT Malaria Ag Pf (pLDH/HRPII)), and *P. vivax* by LDH (BIOCREDIT Malaria Ag Pf/Pv (pLDH/ pLDH)). 75/1060 individuals tested positive for *P. falciparum*, 74/75 of them tested positive for both sample types (1 was negative by capillary blood), and two samples differed by LDH/HRP2 status. 145/1060 samples tested positive for *P. vivax*, with one sample detected only on venous blood. All RDTs were more sensitive than microscopy conducted at the health centers. Microscopy missed 27% (20/74) of *P. falciparum* infections, and 20% (29/145) *P. vivax* infections. Further, microscopy misdiagnosed the incorrect xspecies in 15.4% (29/188) of all microcopy-positive infections. In conclusion, RDTs can be used on capillary and venous blood with no differences in sensitivity.

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SEVERE *PLAMODIUM VIVAX* MALARIA WITH ACUTE KIDNEY INJURY REQUIRING HEMODIALYSIS AND RETINAL INVOLVEMENT A CASE REPORT

Larissa Rocha Alipio[1], Stella Pradebon Silva[1], Jéssica Rafaela dos Santos Alves[2], Ricardo Augusto Chaves de Carvalho[1]

[1]Função De Medicina Tropical Dr Heitor Vieira Dourado, Manaus, Brazil, [2]UNICAMP, São Paulo, SP, Brazil

Although *Plamodium vivax* has traditionally been considered a benign infection, growing evidence highlights its potential for severe and life-threatening complications. Reports of multi-organ dysfunction and atypical manifestations, including retinal involvement, remain limited. We report a 37-year-old woman from the Brazilian Amazon with type 2 diabetes mellitus and hypertension who developed severe *P. vivax* malaria after exposure in an endemic area. She initially presented with fever, vomiting, and asthenia, progressing to jaundice, oliguria, and altered mental status. Diagnosis was confirmed by thick blood smear (+++), and oral treatment failed due to persistent vomiting. On admission to a tertiary center, she exhibited severe multi-organ dysfunction, including thrombocytopenia (36,890/mm^3), acute kidney injury (creatinine 9.2 mg/dL; eGFR 5 mL/min/1.73m^2), hyperbilirubinemia (12.73 mg/dL), and electrolyte abnormalities. She was treated with intravenous artesunate and required urgent hemodialysis due to anuric renal failure. The patient showed marked clinical improvement, with recovery of renal function, normalization of platelet count, and resolution of neurological symptoms. Parasite clearance was confirmed by day 3, allowing initiation of primaquine. After completing treatment, she developed acute bilateral visual impairment. Neuroimaging was unremarkable, but fundoscopic examination revealed retinal hemorrhages and cotton wool spots in the right eye, with proliferative diabetic retinopathy bilaterally. This case underscores the severity of *P. vivax* infection, including rare complications such as dialysis-requiring acute kidney injury and retinal involvement. While malarial retinopathy is well described in *P. falciparum*, it remains poorly characterized in *P. vivax*. Comorbidities such as diabetes and hypertension may contribute to worse outcomes. Early recognition and aggressive management are essential. Retinal evaluation should be considered in patients with visual symptoms, as ocular complications may lead to permanent impairment.

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CLOSING THE DIAGNOSTIC GAP FOR MALARIA ELIMINATION: FIELD IMPLEMENTATION OF MOLECULAR DIAGNOSTIC TOOLS TO DETECT SUBMICROSCOPIC INFECTIONS IN HARD-TO-REACH AMAZONIAN COMMUNITIES

Susan Paredes-Fernandez[1], Jesus Risco-Glaston[2], Rodrigo Villa-Díaz[1], Angie Rojas-Alván[2], Viviana Pinedo-Cancino[2], **Christopher Delgado Ratto**[1]

[1]University of Antwerp, Antwerp, Belgium, [2]Universidad Nacional de la Amazonía Peruana, Iquitos, Peru

The detection of submicroscopic malaria infections, most of them asymptomatic, remains a major challenge for malaria elimination in the Peruvian Amazon, where they represent up to 84% of reported cases by the Ministry of Health (MoH). Routine diagnosis using light microscopy or rapid diagnostic tests (RDTs) underestimates the true burden of infection, thereby sustaining transmission despite temporary declines in case counts. Bridging the gap between molecular diagnostics and their field implementation is critical to strengthen malaria elimination strategies, particularly in settings with a high prevalence of submicroscopic infections. We evaluated the diagnostic performance and operational feasibility of three loop-mediated isothermal amplification (LAMP) protocols in resource-limited Amazonian settings in Loreto, Peru. First, LAMP protocols were deployed at the hospital in Nauta (90 km from Iquitos) using 20 passively detected symptomatic cases to assess performance under routine clinical conditions. Second, 284 samples were collected through reactive case detection in the rural community of Intuto (170 km from Iquitos; ~18 hours by boat), where local MoH staff used two LAMP protocols at point-of-care to evaluate decentralized implementation, diagnostic yield, and user-level performance. Third, ongoing evaluation in Pampa Hermosa (300 km from Iquitos) applies a seek-and-treat strategy to assess the impact of repeated interventions on malaria incidence.Results from Phases I and II showed that LAMP had higher sensitivity than light microscopy, identifying more than twice as many infections. Across phases, LAMP demonstrated feasibility in remote settings, despite operational constraints, and improved detection of hidden parasite reservoirs compared to standard methods. These findings support the integration of molecular diagnostics into routine surveillance strategies to enhance malaria elimination efforts.

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DETECTION OF LOW-DENSITY *PLASMODIUM VIVAX* AND *P. FALCIPARUM* INFECTIONS USING AN IN-HOUSE COLORIMETRIC LAMP ASSAY FROM DRIED BLOOD SPOTS IN ACTIVE AND PASSIVE CASE DETECTION

Oscar Nolasco[1], Joaquín Gomez[2], Hugo Rodriguez-Ferrucci[3], Dionicia Gamboa[1], **Christopher Delgado Ratto**[2]

[1]Universidad Peruana Cayetano Heredia, Lima, Peru, [2]University of Antwerp, Antwerp, Belgium, [3]Universidad Nacional de la Amazonía Peruana, Iquitos, Peru

In the Americas, many countries face a high prevalence of low-density malaria infections that remain undetected by microscopy and rapid diagnostic tests (RDTs). Moreover, WHO-recommended strategies targeting the parasite reservoir, such as "seek and treat" approaches, still rely on these limited diagnostic tools. National malaria elimination programs have therefore called for the development and evaluation of field-deployable diagnostic methods capable of detecting submicroscopic infections in resource-constrained settings. The use of such tools in population-level interventions could substantially reduce the silent reservoir of asymptomatic infections and accelerate progress towards elimination. Polymerase chain reaction (PCR), the reference diagnostic method in research, provides high accuracy but requires infrastructure often unavailable in rural endemic settings. Loop-mediated isothermal amplification (LAMP) offers a sensitive and field-adaptable alternative. We evaluated the diagnostic performance of an in-house colorimetric LAMP assay (cLAMP) using dried blood spot (DBS) samples collected through Active Case Detection (ACD) and Passive Case Detection (PCD) in a remote Amazonian community. Using PCR as reference, we compared cLAMP with boil-and-spin (LBS) and column-based extraction (LCS) against microscopy. LCS-cLAMP showed significantly higher discrimination (AUC) than microscopy in PCD (0.91 vs 0.86; p=0.033) and ACD (0.91 vs 0.78; p<0.001). LBS-cLAMP showed modest, non-significant improvements in ACD (0.82 vs 0.78; p=0.125) and PCD (0.88 vs 0.86; p=0.146). cLAMP combined with high-quality DNA extraction improves detection of low-density infections. The use of DBS enhances feasibility by overcoming logistical constraints of whole blood. cLAMP represents a flexible molecular tool with higher diagnostic performance than microscopy and faster turnaround than PCR. Future work should optimize simplified extraction methods and evaluate field implementation with national malaria programs.

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LONGITUDINAL DYNAMICS OF *PLASMODIUM* POSITIVITY AND GAMETOCYTE BURDEN ACROSS EIGHT SENTINEL SITES IN KENYA (2025)

Redemptah Yeda[1], Raphael Onyango Okoth[2], Benjamin Opot[3], Agnes Chelangat Cheruiyot[4], Edwin Mwakio[5], Dennis Wekesa Juma[6], Hoseah Akala[7]

[1]United States Army Medical Research Institute- Africa, Kisumu, Kenya, [2]HJFMRI, Kisumu, Kenya, [3]United States Army Medical Research Unit - Kenya, Nairobi, Kenya, [4]Kenya Medical Research Institute/ USAMRU-K, Kisumu, Kenya, [5]Kenya Medical Research Institution, Kisumu, Kenya, [6]United States Army Medical Research Directorate-Kenya, Kisumu, Kenya, [7]USAMRU-K, Kisumu, Kenya

This study investigated *Plasmodium* prevalence and gametocyte carriage across eight ecologically diverse regions of Kenya in 2025—Busia, Kericho, Kisumu, Kombewa, Kisii, Marigat, Isiolo, and Lanet. Longitudinal clinical samples were collected at baseline (Day 0) and on Days 3, 7, 14, 21, 28, and 42 to evaluate parasite positivity and gametocyte burden over time. Diagnosis was performed using microscopy and realtime PCR, while gametocytes were quantified to assess transmission potential. Baseline results revealed substantial geographic variability in malaria burden. Kisumu (n=131) and Kombewa (n=119) recorded the highest *Plasmodium* positivity, confirming them as hightransmission hotspots. Moderate prevalence was observed in Busia and Marigat (each n=61), while Kericho and Kisii showed lower but notable positivity. Isiolo (n=6) and Lanet (n=3) had the lowest baseline prevalence. Baseline gametocyte burden followed a similar pattern, with Kisumu (n=65) and Kombewa (n=54) showing the highest levels, while Lanet reported zero gametocytes. After treatment—presumed to be ACT—a sharp decline in parasite positivity occurred by Day 3, with most sites reporting complete clearance. Exceptions included Kisumu (n=21) and Kombewa (n=6), likely reflecting higher initial parasitemia or rapid reinfection in hightransmission settings. By Day 7, however, all sites experienced a resurgence of positivity. Kisumu (n=48), Kombewa (n=44), and Marigat (n=37) exhibited the strongest rebounds. Gametocytes also reappeared, especially in Marigat (n=4) and Kisumu (n=3), suggesting renewed transmission potential. Late follow-up (Days 14-42) showed persistent low-level infections, with Marigat demonstrating gametocyte carriage up to Day 28 (n=5). These findings highlight Kenya's heterogeneous malaria landscape and underscore the need for targeted interventions, improved follow-up, and strengthened vector control to address reinfection and residual transmission

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PERSISTENT NON-FALCIPARUM MALARIA IN KENYA: EVIDENCE FROM 17 YEARS OF SENTINEL SURVEILLANCE

Maurine Atieno Mwalo[1], Gladys Chebet Chemwor[2], Raphael Onyango Okoth[2], Benjamin Opot[3], Edwin Mwakio[4], FARID SALIM ABDI[5], Redemptah Yeda[6], Risper Nyaboke Maisiba[7], Agnes Chelangat Cheruiyot[2], Christopher Ekuttan[8], Milton Obilo[8], John Lugonzo[8], Rebecca Mutembula[8], Juliana Munyao[8], Charles Okudo[1], Dennis Wekesa Juma[9], Robert Haynes[10], Luis Powsang[10], Hoseah Akala[6]

[1]WRAIR-A/KEMRI, Kisumu, Kenya, [2]HJFMRI, Kisumu, Kenya, [3]United States Army Medical Research Unit - Kenya, Nairobi, Kenya, [4]Kenya Medical Research Institution, Kisumu, Kenya, [5]Walter Reed, KISUMU, Kenya, [6]USAMRU-K, Kisumu, Kenya, [7]Kisumu, Kenya, [8]KDF, Nairobi, Kenya, [9]United States Army Medical Research Directorate-Kenya, Kisumu, Kenya, [10]WRAIR-A, Kisumu, Kenya

Although eight *Plasmodium* species infect humans, *P. falciparum* is the most virulent and, therefore, at the center of most interventions. In sub-Saharan Africa, mixed species infections are common, with nearly all possible species compositions observed. The latter may be more challenging to diagnose, treat, control, and ultimately eliminate. Therefore, effective eradication of malaria requires comprehensive detection and estimation of the burden of parasite species during intervention enforcement. This study aimed to establish the burden of *Plasmodium* species among symptomatic individuals in selected regions of Kenya between 2008 and 2025. Between 2008 and 2025**,** a total of 4,255 blood samples were collected from symptomatic individuals presenting at healthcare facilities across 7 sentinel sites, namely, Busia, Kericho, Kisii, Kisumu, Kombewa, Malindi, and Marigat, and were analyzed for species composition via qPCR. Across all sites and years, *P. falciparum* was the dominant species 4,139/4,255 (97.3%), besides *non-falciparum* species, including *P. ovalewallikeri* 545/4,255 (12.8%), *P. malariae* 235/4,255 (5.5%), and *P. ovalecurtisi* 217/4,255 (5.1%). up to 3,460/4,255 (81.3%) were single-species infections. *Plasmodium falciparum* consistently remained the dominant malaria species across all study sites, while mixed species infections involving *P. malariae, P. ovalecurtisi*, and *P. ovalewallikeri* occurred intermittently at low frequencies, indicating sustained but limited transmission of *non-falciparum* species. In conclusion**,** hough *Plasmodium falciparum* remained the predominant species, the continued detection of *non-falciparum* parasites—including *Plasmodium malariae*, *P. ovalecurtisi,* and *P. ovalewallikeri* and the occurrence of mixed infections indicate persistent low-level transmission of these species. These findings highlight the importance of sensitive multi-species diagnostic and surveillance approaches to better inform selection and deployment of malaria interventions to bring down the burden globally.

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SEROLOGICAL BIOMARKERS OF MOSQUITO EXPOSURE REVEAL HIDDEN HETEROGENEITY IN HAITI'S MALARIA ELIMINATION LANDSCAPE

Alyssa R. Schwinn[1], Will Eaton[1], Sara Harris[1], Vena Joseph[2], Alexandre Existe[3], Jacques Boncy[3], Eric Rogier[4], Michelle A. Chang[4], Daniel Impoinvil[4], Ruth A. Ashton[1], Thomas Druetz[2], Thomas P. Eisele[1], Berlin Londono-Renteria[1]

[1]Tulane University, New Orleans, LA, United States, [2]Université de Montréal, Montréal, QC, Canada, [3]Laboratoire National de Santé Publique, Port-au-Prince, Haiti, [4]Centers for Disease Control & Prevention, Atlanta, GA, United States

Anopheles albimanus (*Nyssorhynchus*) is the main malaria vector on Hispaniola. However, five other *Anopheles* species have been reported circulating in the area; four belonging to the subgenus *Anopheles* (*An. crucians*, *An. grabhamii*, *An. pseudopunctipennis*, and *An. vestitipennis*) and one to the *Nyssorhynchus* subgenus (*An. argyritarsis*). Previous studies have identified immunogenic salivary peptides, several unique to the *Nyssorhynchus* and *Anopheles* subgenera, underscoring their potential as biomarkers for differentiating exposure in both Old and New World anophelines. Since both subgenera have been reported in Haiti, ELISAs were conducted to quantify IgG antibody titers against three antigenic anopheline salivary peptides (gSG6-P1, Peroxi-P3, and Apy-2) in 348 participants enrolled in Haiti's multidisciplinary Malaria Zero Program. This study aimed to evaluate the intensity of human-vector contact with *Anopheles* from both subgenera in Grand' Anse, Haiti, and to assess associations between anti-parasite and anti-peptide antibodies. Significantly elevated IgG responses to Peroxi-P3 compared to Apy-2 and gSG6-P1 ($p < 0.001$) were observed. Responses to Peroxi-P3 and gSG6-P1 differed significantly between ≤18-year-olds and >18-year-olds ($p = 0.004$ and $p = 0.002$), whereas no sex-based differences were observed. Correlation analyses showed more significant positive associations between gSG6-P1 and Plasmodium antigens than any other peptide, particularly among ≤18-year-olds. A marked reduction in IgG responses to Apy-2 and Peroxi-P3, but not gSG6-P1, was observed among participants with a single household animal species compared with those with multiple or no animals. Lastly, spatial analysis revealed heterogeneous geographic overlap of high antibody responses across peptides, alongside clusters of low responses to Peroxi-P3 and Apy-2. These results provide additional evidence supporting the utility of anopheline salivary peptides to characterize human-vector-parasite exposure dynamics in low-transmission settings such as Haiti.

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DETERMINANTS OF MALARIA MORTALITY IN A REGIONAL HOSPITAL IN BURKINA FASO: A RETROSPECTIVE ANALYSIS OF 80 DEATHS TO IMPROVE CARE

Yacouba Belem[1], Aissata Barry[2], Ousmane Badolo[1], Boukary Bance[2], Boulaye Dao[2], Boureima Ouedraogo[1], Ali Sogli[3], André Koné[1], Gladys Tetteh[4]

[1]Jhpiego, Ouagadougou, Burkina Faso, [2]SP/Palu, Ouagadougou, Burkina Faso, [3]Djoro Regional Health Directorate, MoH, Gaoua, Burkina Faso, [4]Jhpiego, Washington, DC, United States

Malaria accounted for 43.9% of hospitalizations and 8.7% of deaths in Burkina Faso in 2024. (MoH, statistical yearbook 2024). At the Gaoua Regional Hospital, in the same period, the fatality rate was 4.4% higher than the national average (1,3%). During the first 48 weeks of 2025, the hospital recorded a mortality rate of 5.34% among children under the age of 5, which remained above the expected threshold of 0.5%, suggesting critical gaps in case management. This investigation aimed to identify critical health system and clinical factors associated with malaria-related deaths and propose measures to improve care. The study consisted of a descriptive retrospective review covering the first 48 weeks of 2025 and examining all 80 medical records of patients who died from malaria identified in the treatment units. The review was completed by interviews with the heads of the care units. The results indicate that 74% of the deaths involved children under five years of age, and 86% of the deceased patients resided in rural areas. Of all deceased patients, 73.8% were referred from health facilities, and among them, 38% had received inadequate pre-transfer care. Additionally, more than 50% were evacuated by non-medical transport. Regarding the quality of care provided, 27.6% of patients requiring a transfusion did not receive one, and the administration times for treatments, particularly injectable artesunate, were not followed according to protocol. The analysis also revealed the absence of a definitive diagnosis recorded in the medical records, leading to erroneous reports attributing the deaths to malaria (A total of 7 deaths attributed to malaria that do not meet the case definition). Malaria-related mortality was driven by the gaps in the referral system, delays in care, organizational failures in the administration of treatment, and errors in the final classification of deaths attributable to malaria. The study recommends a coordinated strengthening of skills, healthcare organization, and the information system through Coaching, mentoring, supportive supervision and the provision of supplies including blood products.

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MALARIA RESURGENCE AND RAPID RE-ELIMINATION IN COSTA RICA (2020-2025): A MOBILITY-DRIVEN EPIDEMIC IN A NEAR-ELIMINATION SETTING

Jennyffer Gonzalez[1], Rodrigo Marin[1], Isaac Vargas[1], Sara Arce[1], Veronica Cruz[2], Elena Rodriguez[2], Emma Margarita Iriarte[3], Yadel Centeno[2], Leopoldo Villegas[4]

[1]Ministry of Health, San Jose, Costa Rica, [2]Consultant IREM, San Jose, Costa Rica, [3]Inter American Development Bank-IDB, Washington, DC, United States, [4]International Advisor Independent, Silver Spring, MD, United States

Costa Rica, historically near malaria elimination, experienced a resurgence driven by cross-border mobility and focal transmission. A retrospective analysis of national surveillance data (2020-2025) characterized epidemiological trends, including species distribution, case classification, demographic patterns, and foci dynamics, as well as diagnostic effort and intervention scale-up. Between 2021 and 2023, a focal outbreak in Los Chiles, a northern border canton, accounted for the majority of cases, reflecting intense transmission linked to cross-border mobility and occupational exposure. Malaria cases peaked in 2023 (n=520 *Plamodium falciparum*) and declined sharply to 21 in 2025 and to 27 by early 2026, representing a 96% reduction following an intensified response. Transmission remained highly focal, with five active foci identified in 2026. Cases were concentrated among adult males (74%), predominantly Nicaraguan migrants (92%), and miners (63%), highlighting the role of mobile, high-risk populations. Imported and introduced cases varied by year, with transmission closely associated with northern border regions and mining areas. Diagnostic effort increased substantially, reaching 162,241 tests in 2025, supporting early detection and rapid response. Intensified interventions—including active surveillance, targeted screening, and deployment of field personnel in high-risk areas—were temporally associated with rapid interruption of transmission, despite continued cross-border movement. Malaria resurgence in Costa Rica was driven by a mobility-linked epidemic but rapidly reversed through targeted interventions and strengthened surveillance. Sustaining elimination will require institutionalizing strategies addressing cross-border mobility, occupational exposure, and focal transmission in high-risk populations.

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TANZANIA'S HIDDEN MALARIA TIME BOMB: ASYMPTOMATIC SCHOOL-AGED CHILDREN RESERVOIR UNCOVERED

Sarah Leonard Rumisha[1], Frank Chacky[2], Susan Fred Rumisha[3], Punam L. Amratia[1], Sosthenes Charles Ketende[4], Pendael Machafuko[5]

[1]Ifakara Health Institute, Dar es Salaam, United Republic of Tanzania, [2]Ministry of Health (MoHCDGEC), Natl Malaria Control Programme, Dar

Es Salaam, United Republic of Tanzania, [3]Malaria Atlas Project, Dar Node, Ifakara Health Institute, United Republic of Tanzania, [4]Dar es Salaam, United Republic of Tanzania, [5]National Institute for Medical Research, Tanga, United Republic of Tanzania

School-aged children (SAC, 5-16 years) bear a disproportionate yet largely unaddressed malaria burden in Tanzania, acting as a major asymptomatic reservoir sustaining transmission. In routine practice, fever is often the primary trigger for care-seeking at household and malaria testing at health facilities, meaning infections without fever may remain undetected. Understanding the burden and distribution of asymptomatic malaria carriers is critical for elimination progress. This study assessed the distribution and burden of asymptomatic malaria across age, sex, and spatial heterogeneity using data from a nationally representative survey conducted in 2023 where 64,033 SAC were tested for malaria using Rapid Diagnostic Tests (RDTs) and checked for presence of fever, body temperature ≥37.5°C. Symptomatic malaria was defined as RDT-positive with fever while asymptomatic malaria as RDT-positive without fever. Overall malaria prevalence in SAC was 11.2% amongst these 86.2% coming from the asymptomatic group. Males (10.7%) consistently showed higher asymptomatic prevalence than females (8.6%), and prevalence increased with age, 7.6% in 5-8 years and peaking among adolescents (13-16 years) with 12.2%. Asymptomatic infections dominate in 81% of the councils [Top 3 - Nyang'wale DC, Bukombe DC and Ushetu DC], 13.6% had an equal split with most of these being low strata councils [Top 3 - Mbulu DC, Bukoba MC, Chamwino DC]. Only a small proportion of councils, 5.4%, had predominantly symptomatic malaria SAC [Top 3 - Nanyamba TC, Geita TC, Msalala CD]. These patterns highlight substantial geographic heterogeneity and the widespread of asymptomatic reservoirs, underscoring the need for targeted and stratified interventions for SAC to reduce transmission and attain Tanzania's 2030 elimination goals.

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MOLECULAR ANALYSIS REVEALS HETEROGENEITY OF *PLASMODIUM FALCIPARUM* INFECTIONS BY REGION AND TRANSMISSION INTENSITIES IN MAINLAND TANZANIA

Salehe S. Mandai[1], Happyness A. Mwailunga[1], Hussein J. Semboja[1], Rule B. Mrengela[1], Laurencia S. Tangawizi[1], Gideoni I. Maulidi[1], Baraka W. Mbuba[1], Catherine B. Mvaa[1], Angelina J. Kisambale[1], Dativa Pereus[1], Doris D. Mbata[1], Ninja S. Bisaga[2], Daniel P. Challe[3], Daniel A. Petro[4], Solomon M. Mwakasungula[1], Rashid A. Madebe[2], Daniel B. Mbwambo[5], Sijenunu Aaron[5], Abdallah Lusasi[5], Samwel Lazaro[5], Stella Kajange[6], Celine I. Mandara[2], Misago D. Seth[2], Deus S. Ishengoma[1]

[1]Ifakara Health Institute, Dar es Salaam, United Republic of Tanzania, [2]National Institute for Medical Research, Dar es Salaam, United Republic of Tanzania, [3]National Institute for Medical Research, Tanga, United Republic of Tanzania, [4]University of Dar es Salaam, Dar es Salaam, United Republic of Tanzania, [5]National Malaria Control Program, Dodoma, United Republic of Tanzania, [6]President's Office, Regional Administration and Local Government, Dodoma, United Republic of Tanzania

In Tanzania, malaria diagnosis in routine settings relies on rapid diagnostic tests (RDTs), which provide limited information beyond a positive result. In moderate and high-transmission areas, antigen persistence may lead to an overestimation of active infections. Consequently, routine surveillance data may not accurately reflect true infection patterns across transmission strata. Molecular analysis of routinely collected samples can be used to confirm infections and provide additional epidemiological information. In this study, we analysed RDT-positive samples collected between March and December 2025 among outpatients from 92 health facilities in 26 regions of Mainland Tanzania. 14,183 samples were collected as dried blood spots, and infections of *Plasmodium* spp were confirmed using quantitative PCR (qPCR). At present, 5,394 samples from 10 regions have been analysed, of which 91.0% (n=4906) were confirmed as malaria-positive by qPCR. Significant regional variation was observed ($p<0.001$), with the *Plasmodium falciparum* positivity rate by qPCR ranging from 85.7% to 98.6%. Differences were also observed across transmission strata ($p<0.001$). However, *P. falciparum* was detected in a high proportion of confirmed infections across all settings, with higher proportions in low-transmission areas (96.0%) compared to other strata (89.2-91.2%). Parasite density varied across transmission strata and was higher in low and very-low-transmission areas (~1,415 parasites/μL) compared with moderate and high-transmission areas (~670 p/μL). These findings show that most cases represent true *P. falciparum* infections and vary geographically. Higher parasite densities in low-transmission areas and lower confirmation rates in higher transmission settings may reflect differences in infection dynamics, including possible residual antigen detection. Molecular analysis of routinely collected samples could strengthen malaria surveillance by providing a practical approach to validate routine diagnoses and capture epidemiological variations in malaria burden.

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DRY-SEASON AGE-SPECIFIC DYNAMICS OF ASYMPTOMATIC *PLASMODIUM FALCIPARUM* INFECTION IN RURAL MALI: SEVEN YEARS OF LONGITUDINAL EVIDENCE

Daouda Sanogo[1], Mahamoudou Toure[1], Soumba Keita[2], Fousseyni Kane[3], Moussa Keita[4], Ibrahim Sanogo[5], Drissa Konate[6], Hamady Coulibaly[1], Sory Ibrahima Diawara[1], Nafomon Sogoba[7], Mahamadou Diakite[8], Seydou Doumbia[9]

[1]University of Sciences, Techniques and Technologies (USTTB), Bamako, Mali, [2]Bamako, Mali, [3]UCRC - ICERMali- USTTB, Bamako, Mali, [4]Intl Ctr for Excellency in Res/Malaria Res & Training Ctr, Bamako, Mali, [5]University Clinical Research Center, Bamako, Mali, [6]malaria research and training center, Mali, [7]ICER-MRTC-Entomology, Bamako, Mali, [8]MRTC-USTTB, Bamako, Mali, [9]University Clinical Research Center, Faculty of Medicine, BAMAKO, Mali

As malaria transmission declines in sub-Saharan Africa, understanding the persistence and seasonal dynamics of asymptomatic *Plasmodium falciparum* infections is critical for targeting residual transmission. School-age children are increasingly recognized as an important reservoir, yet their contribution across dry seasons remains poorly defined. This study assessed age-specific patterns of parasite carriage across the long dry season in a high-transmission setting in Mali.Longitudinal cross-sectional surveys were conducted in Dangassa, Mali, at the end of the transmission season (November) and at the beginning of the subsequent season (June) between 2014-2021. Asymptomatic infections were defined as microscopy- or rapid diagnostic test-positive *P. falciparum* in afebrile individuals without recent fever or treatment. Paired seasonal observations were used to estimate age-specific frequencies of persistent infection, clearance, new infection, and sustained negativity. From 2014 to 2021, asymptomatic prevalence declined significantly from 55.5% to 5.3% at the start of the rainy season, with similar reductions observed at season end. Children aged 5-14 years consistently exhibited the most intense infection dynamics across seasons, with the highest persistence (Pos→Pos 78.4% vs 40.5% in adults), highest acquisition (Neg→Pos >69.4 % vs 28.4% in adults), and the lowest stability of uninfected status (Neg→Neg as low as 30.6 % vs >71.7% in adults) during the rainy season. During the dry season, although overall transmission was low, persistence remained highest in this group (Pos→Pos 46.1 % vs 20.2% in adults) confirming a disproportionate contribution of school-age children to maintaining the parasite reservoir. Despite substantial declines in transmission, school-age children remain the main reservoir of asymptomatic infection across seasons, supporting their prioritization in strategies targeting residual malaria transmission.

6697

INFERRING THE RELATIONSHIP BETWEEN THE PREVALENCE OF MALARIA INFECTIONS AND CASE INCIDENCE USING ROUTINE SURVEILLANCE DATA

Daniel A. Petro[1], Sijenunu Aaron[2], Frank Chacky[2], Samwel Lazaro[2], Kien T. Tran[1], Sarit Adhikari[1], Chaoyi-Yi T. Chao[1], Happyness A. Mwailunga[3], Baraka W. Mbuba[3], Dativa Pereus[4], Ninja S. Bisaga[5], Catherine Bakari[3], Rule Budodo[3], Salehe S. Mandai[3], Laurencia S. Tangawizi[3], Gideoni I. Maulidi[3], Hussein J. Semboja[3], Angelina J. Kisambale[3], Doris Mbata[5], Daniel P. Challe[6], Gervas A. Chacha[5], Solomon M. Mwakasungula[3], Rashid A. Madebe[5], Stella Kajange[7], Celine I. Mandara[5], Misago D. Seth[5], Deus S. Ishengoma[3], Maciej F. Boni[1]

[1]Temple University, Philadelphia, PA, United States, [2]National Malaria Control Programme, Dodoma, United Republic of Tanzania, [3]Ifakara Health Institute, Dar es Salaam, United Republic of Tanzania, [4]Muhimbili University of Health and Allied Sciences, Dar es Salaam, United Republic of Tanzania, [5]National Institute for Medical Research, Dar es Salaam, United Republic of Tanzania, [6]National Institute for Medical Research, Tanga, United Republic of Tanzania, [7]Prime Minister's Office, Regional Administration and Local Government, Dodoma, United Republic of Tanzania

Ecological dynamics and infectious disease interventions strongly shape the relationship between disease incidence and prevalence. The context-dependence of these complementary measures of disease burden means that their relationship is rarely constant. For malaria, there is limited evidence on whether the prevalence-incidence (PI) relationship in the post-artemisinin-based combination therapy (ACT) era tends to saturate upward or shift to the right. For our study, we used routine prevalence surveillance data from pregnant women attending their first antenatal care (ANC) visits and confirmed cases reported by all health facilities in 184 councils across Mainland Tanzania between 2014 and 2024 to investigate how ecological variations and intervention contexts regulate the PI relationship. National and council-specific PI curves were estimated using a nonlinear mixed-effects model with council-level random effects and temporal correlation. A nonlinear PI relationship that saturated to the right was observed, with a significant change in slope at 114.3 cases per 1000 persons per year [PPY] (95% CI: 105.4 - 123.2; p<0.001), decreasing from 0.094 to 0.011 (p<0.001). Across most councils, a one-percentage-point increase in ANC prevalence corresponded to approximately 15 (interquartile range: 10 - 21) additional malaria cases per 1000 PPY. About 44.6% (82/184) of the councils had higher asymptotic ANC prevalence than the estimated national average of 17.2% (95% CI: 14.9 - 19.9%), while 52.7% (97/184) had an incidence threshold exceeding the national average of 107.2 cases per 1000 PPY (95% CI: 82.7 - 139.1). An increase in the proportion of confirmed cases treated with ACT and maximum temperature reduced the upper bound of detectable ANC prevalence, while higher vegetation cover and rainfall were associated with a steeper (higher initial slope) PI curve. Similar incidence patterns generated different ANC prevalence levels across councils, highlighting the need of accounting for ecological and intervention-driven heterogeneity when interpreting malaria surveillance data.

6698

CLINICAL *PLASMODIUM FALCIPARUM* AND *P. VIVAX* INFECTION IN THE ETHIOPIAN HIGHLANDS ARE OBSERVED ACROSS SEASONS AND RARELY RELATED TO TRAVEL

Laurel Anne Lown[1], Yalemwork Ewnetu[2], Lise Carlier[3], Wossenseged Lemma[2], Nega Berhane[2], Cristian Koepfli[1]

[1]University of Notre Dame, Notre Dame, IN, United States, [2]University of Gondar, Gondar, Ethiopia, [3]LMC Projects, Amsterdam, Netherlands

The Ethiopian highlands were historically believed to be malaria free, yet clinical cases are frequently observed in health centers >2000 m. The proportion of cases that recently returned from the lowlands where malaria is endemic is poorly described. Febrile patients were enrolled from November 2019 to September 2020 in a health center in Gondar, Ethiopia, at 2100 m. *Plasmodium falciparum* and *P. vivax* were detected by microscopy conducted at the health center, and by qPCR. A total of 1819 participants were enrolled. Test positivity by microscopy and qPCR was 58.1% and 64.1% for *P. falciparum*, and 41.8% and 35.9% for *P. vivax*. Only 3.1% of individuals reported travel to the lowlands within 4 weeks prior to sample collection. Travel increased the odds of malaria infection by 5.2 times, but 92.4% of individuals who tested positive did not report travel. Adults 16-35 years of age accounted for 57% of individuals who presented for testing and positivity peaked in this age group (*P. falciparum*: 27.3%, *P. vivax*: 10.9%). Test positivity of males was almost twice as high as in females. No age trends in parasite densities were observed. Most individuals who were malaria positive had not traveled, suggesting that local transmission is occurring within the Ethiopian highlands. The concentration of infections among adult males points to a strong behavior-related risk of infection.

6699

UPTAKE OF TREATMENT-SEEKING BEHAVIORS FOR MALARIA DURING PREGNANCY AND ADVERSE CLINICAL DIAGNOSES: A CROSS-SECTIONAL STUDY IN BIOKO ISLAND, EQUATORIAL GUINEA

Madysen Schreiber[1], Jordan Smith[2], David Galick[3], Jeremias Nzamio Mba Eyono[3], Norberto Bosepa Cubacuba[3], Miriam Riobi Patabobe[3], Wonder Philip Phiri[3], Matilde Riloha Rivas[4], Guillermo Alberto Garcia[2], Carlos Guerra[2], Emily Henkle[1], Deborah Karasek[1], Nicole I. Wakim[1], Eric Hall[1]

[1]Oregon Health & Science University-Portland State University School of Public Health, Portland, OR, United States, [2]MCD Global Health, Silver Spring, MD, United States, [3]MCD Global Health, Malabo, Equatorial Guinea, [4]Ministerio De Salud, Malabo, Equatorial Guinea

Intermittent preventive treatment in pregnancy (IPTp) and antenatal care (ANC) attendance are key malaria prevention strategies for pregnant women in sub-Saharan Africa. Despite increased ANC attendance, IPTp uptake remains suboptimal, and the implications for maternal morbidity during pregnancy are not well characterized. We evaluated associations between IPTp uptake, ANC attendance, and adverse clinical diagnoses during pregnancy on Bioko Island, hypothesizing that adherence to optimal IPTp and ANC schedules are associated with reduced adverse clinical diagnoses. We analyzed routinely collected ANC data from public health facilities from 2015-2023. IPTp uptake and ANC attendance were classified as optimal or non-optimal based on gestational-age-specific World Health Organization recommendations. Outcomes included maternal bacterial, viral, and parasitic infection diagnoses, and anemia. Log-binomial regression estimated adjusted prevalence ratios (aPRs), controlling for maternal age, district, gestational age, and malaria season, with assessment of effect modification by malaria infection status. A total of 103,759 ANC visits were included in IPTp analyses and 49,668 in ANC attendance analyses. Non-optimal IPTp uptake was associated with higher prevalence of bacterial (aPR: 1.41, 95% CI: 1.34, 1.47), viral (aPR: 2.22, 95% CI: 2.02, 2.43), and parasitic infections (malaria aPR: 2.54, 95% CI: 2.37, 3.72; non-malaria aPR: 1.33, 95% CI: 1.16, 1.51). Non-optimal ANC attendance was similarly associated with increased prevalence of bacterial, viral, and malaria infections, as well as maternal anemia (aPR: 1.72, 95% CI: 1.47, 2.01). Effect modification by malaria infection status was observed for IPTp uptake and anemia and for ANC attendance with bacterial and viral infections. In this high-transmission setting, suboptimal engagement with malaria prevention during pregnancy was associated with increased maternal morbidity, highlighting missed opportunities for prevention within routine ANC. These findings support integrating strategies that improve both access and retention of malaria prevention care.

6700

CLINICAL PREDICTORS OF *PLASMODIUM* PARASITES AMONG MALARIA CASES IN SELECTED REGIONS OF MAINLAND TANZANIA

Daniel Protasy Challe[1], Daniel Anderson Petro[2], Filbert Francis[1], Misago Dimson Seth[3], Rashid Ally Madebe[3], Angelina Kisambale[4], Dativa Pereus[4], Salehe Mandai[4], Catherine Bakari[4], Hussein Semboja[4], Solomon Mickson Mwakasungula[4], Gervas Chacha[5], Rule Budodo[4], Baraka Mbuba[4], Happyness A. Mwailunga[4], Gideoni I. Maulidi[4], Laurencia S. Tangawizi[4], Daniel Mbwambo[6], Sijenunu Aaron[6], Abdallah Lusasi[6], Stella Kajange[7], Celine I. Mandara[5], Vedastus W. Makene[8], Deusdedith R.S. Ishengoma[3]

[1]National Institute for Medical Research, Tanga, United Republic of Tanzania, [2]University of Dar es Salaam, Dar es Salaam, Tanzania, Dar es Salaam, United Republic of Tanzania, [3]National Institute for Medical Research, Dar es Salaam, Tanzania, Dar es Salaam, United Republic of Tanzania, [4]Ifakara Health Institute, Dar es Salaam, Tanzania, Dar es Salaam, United Republic of Tanzania, [5]National Institute for Medical Research, Dar es Salaam, United Republic of Tanzania, [6]National Malaria Control Programme, Dodoma, Tanzania, Dodoma, United Republic of Tanzania, [7]Prime Minister's Office, Regional Administration and Local Government, Dodoma, Tanzania, Dodoma, United Republic of Tanzania, [8]Open University of Tanzania, Dar es Salaam, Tanzania, Dar es Salaam, United Republic of Tanzania

Malaria remains a leading public health concern in Tanzania, and it presents with diverse clinical manifestations that often overlap with other febrile illnesses, complicating clinical diagnosis and effective treatment. Understanding the patterns of symptoms and risk factors associated with *Plasmodium* infection is essential for improving case management through prompt and correct diagnosis. A community cross-sectional survey was conducted in 13 villages located in five districts from five regions with varying transmission intensities to assess clinical manifestations and factors associated with *Plasmodium* infections. The study was conducted from June to August 2025 and recruited 7,612 participants with or without malaria symptoms. *Plasmodium* infections were confirmed among 41.9% (n = 3,187) using rapid diagnostic tests (RDTs). Using multivariable logistic regression analysis, participants with a history of fever (prevalence = 66.3% and adjusted odds ratio (aOR)=2.2, 95% confidence interval (CI)=1.7-2.9; p<0.001), measured fever (64.1% and aOR=2.8, 95%CI=2.4-3.3; p<0.001), vomiting (89.8% and aOR=3.8, 95%CI=2.0-7.3; p<0.001) had significantly higher prevalence and odds of infections. While headache was highly prevalent (57.1%), it was associated with significantly reduced odds (aOR=0.7, 95%CI=0.6-0.8; p<0.001). Other symptoms, including convulsions (85.7% and aOR=2.4, 95%CI=0.4-12.8; p=0.317), yellowness of eyes (80.0% and aOR=1.7, 95%CI=0.2-11.6; p=0.602), general body pains (56.4% and aOR=0.9, 95%CI=0.8-1.1; p0.2871), abdominal pain (51.9% and aOR=1.0, 95%CI=0.9-1.2; p=0.629), loss of appetite (70.0% and aOR=0.8, 95%CI=0.6-1.1; p=0.168), and body weakness or malaise (73.1% and aOR=1.1, 95%CI=0.7-1.6; p=0.0494), were also significantly associated with higher prevalence, however the odds of *Plasmodium* infections were not significant. Clinical symptoms, particularly fever, vomiting, and headache, were strong predictors of *Plasmodium* infection. Integrating symptom-based screening with diagnostic testing can improve early detection and management of malaria in endemic settings.

6701

FROM TRAIT-PERFORMANCE CURVES FOR *ANOPHELES STEPHENSI-PLAMODIUM FALCIPARUM* TO TRANSMISSION MODELS OF URBAN MALARIA

Leonardo Souto Ferreira[1], Paul J. Huxley[2], Brandyce St. Laurent[3], Britny L. Johnson[3], Rajesh Sharma[4], Vijay Kohli[4], Michael C. Wimberly[5], Courtney Murdock[3], Mercedes Pascual[1]

[1]New York University, New York, NY, United States, [2]University of York, York, United Kingdom, [3]Cornell University, Ithaca, NY, United States, [4]Ahmedabad Municipal Corporation, Ahmedabad, India, [5]University of Oklahoma, Norman, OK, United States

Rapid urbanization and the spread of the urban-adapted malaria vector *Anopheles stephensi* from Asia into Africa motivate closer examination of how climate variability shapes malaria transmission in cities. Using our recent experimental data, we developed trait-performance surfaces for the joint effects of temperature (T) and relative humidity (RH) on key mosquito and parasite traits and used them to quantify climatic contributions to urban malaria dynamics at seasonal and interannual scales. We formulate a stochastic climate-driven SEIR-type model in which an effective transmission rate incorporates T-RH-dependent mosquito and parasite traits, while larval carrying capacity is governed by a precipitation-driven dynamical equation. The model combines inferred rainfall-response timescales in larval habitat dynamics with a fast-slow approximation that collapses the vector equations into an effective transmission rate. We ask whether this reduced formulation can reproduce seasonal and interannual variability in malaria incidence in Ahmedabad, India, where seasonal epidemics vary strongly across years. We show that temporal resolution of climate drivers is critical for mechanistic inference. With hourly climate inputs, the effective transmission rate peaks about one month before observed cases, consistent with biological delays, whereas monthly inputs shift this peak to after the outbreak. Thus, sub-monthly temperature variation matters for causal interpretation even when the model is fitted to monthly incidence data. The fitted model captures both seasonality and interannual variability over two decades. Using a likelihood-based attribution framework, we find that temperature and relative humidity drive annual seasonality and epidemic timing, whereas rainfall shapes interannual variability. These results show that trait-based, climate-driven models with explicit timescale separation can reproduce key temporal patterns of urban malaria, providing a parsimonious alternative to higher-dimensional coupled human-vector models.

6702

DRIVERS OF PERSISTENTLY HIGH MALARIA INCIDENCE DURING THE LOW TRANSMISSION SEASON IN NANORO AND TENADO DISTRICTS, BURKINA FASO

ANDRE KONE[1], Yacouba Belem[1], Yacouba Ouedraogo[1], Ousmane Badolo[1], Christian Kompaore[2], Barry Aissata[2], Gladys Tetteh[3]

[1]Jhpiego, Ouagadougou, Burkina Faso, [2]NMCP, Ouagadougou, Burkina Faso, [3]Jhpiego, Baltimore, MD, United States

Malaria remains a significant public health concern in Burkina Faso, even during the low transmission season. Weekly surveillance data from January to May 2025 showed unusually high incidence rates in the health districts of Ténado(332 ‰) and Nanoro(414 ‰), far exceeding regional(121‰) and national(72‰) averages, despite strong support from the U.S. Department of State. An investigation was conducted to validate the data, analyze contextual drivers, and propose practical recommendations to reduce malaria. This was a cross-sectional study conducted in a hospital setting. The sample was selected purposively and comprised 8 health facilities reporting malaria incidence rates higher than the two district averages. A tool developed and validated by SP/Palu was digitized using KoboTool to facilitate data collection, storage, and rapid analysis. The investigation involved direct interviews with healthcare providers focusing on their current practices related to malaria prevention, diagnosis, and treatment. Additional data were collected from consultation registers, health facility weekly

reports, and stock records at the level of essential medicines depots. Weekly data confirmed that the malaria epidemic threshold was exceeded in both districts during January and February 2025. However, 13% of malaria cases reported in the registers did not meet the malaria case definition according to the standard definition of malaria cases (national guidelines for the management of malaria). Nevertheless, the recalculated incidence remained high in both districts: 373 ‰ in Nanoro and 283 ‰ in Ténado, compared with an expected annual value of 95 ‰. Artisanal gold mining sites constitutes an aggravating factor. Populations from these sites are not included in the denominator used for incidence calculation, which biases the estimates. Systematic malaria screening of all patients also generated errors in summaries as well as in ENDOS. The findings of this investigation indicate that specific supervision of malaria care needs to be strengthened to ensure compliance with national guidelines on malaria management

6703

TAKING A ROUND TRIP: ASSESSING THE FULL PICTURE OF MALARIA CONNECTIVITY AND IMPORTATION DYNAMICS TO BIOKO ISLAND

David Galick[1], Dianna Hergott[2], Teresa Ondo Mfumu Ayingono[1], Matilde Riloha Rivas[3], Wonder Philip Phiri[1], David L. Smith[2], Guillermo A. Garcia[4], Carlos Alberto Guerra[5]

[1]MCD Global Health, Malabo, Equatorial Guinea, [2]University of Washington, Seattle, WA, United States, [3]National Malaria Control Program, Malabo, Equatorial Guinea, [4]MCD Global Health, Hallowell, ME, United States, [5]MCD Global Health, Quito, Ecuador

Evidence has consistently pointed to malaria importation as one of the main challenges to achieving elimination on Bioko Island. Most of the imported infections presumably come from mainland Equatorial Guinea (EG), where malaria control has been virtually inexistent and transmission intensity remains high. Though evidence for the latter is incomplete and outdated, malaria prevalence in people traveling between mainland EG and Bioko, measured during malaria indicator surveys (MIS) on the island, steadily remains around 25%. Previous work studying malaria connectivity using Bioko MIS data characterized high human mobility between island and mainland districts that helped explain malaria importation dynamics. However, all prior analyses were limited to characterizing the mainland side of importation indirectly through returning travelers, as no contemporaneous prevalence data from mainland EG were available. In 2025, a new strategy aiming at eliminating malaria at national level was launched in EG. This new effort will expand activities from Bioko to the rest of the country. Baseline MIS of a representative sample of the population will be conducted in June and July 2026 in mainland EG followed by the annual MIS in Bioko in August and September. Both surveys include detailed travel histories and malaria prevalence measured by RDT, enabling direct comparison of malaria risk between Bioko residents and the mainland source population for the first time. These concurrent surveys present the first ever opportunity to directly measure malaria connectivity and importation dynamics from both ends of the transmission chain in country.

6704

ANTIBODY RESPONSES TO *ANOPHELES* SALIVARY AND *PLASMODIUM FALCIPARUM* ANTIGENS REFLECT SEASONAL TRANSMISSION BUT NOT INDIVIDUAL BITING EXPOSURE IN WESTERN KENYA

Erastus Kipkosgei Kirwa[1], Bernard N. Kanoi[2], Kiprotich Chelimo[3], Francis Maina Ndungu[4], Steve Myer Taylor[5], Wendy P. O'Meara[6], Christine Franzel Markwalter[7]

[1]Kenya Medical Research Institute, Kisumu, Kenya, [2]Mount Kenya University, Thika, Kenya, [3]Kenya Med Res Inst, Kisumu, Kenya, [4]KEMRI WELLCOME TRUST RESEARCH PROGRAMME, Kilifi, Kenya, [5]Duke University, Durham, NC, United States, [6]Duke Global Health Institute, Durham, NC, United States, [7]Duke Global Health Institute, Carrboro, NC, United States

Monitoring antibody responses to mosquito salivary antigens and *Plasmodium falciparum* proteins offers a promising approach for assessing malaria transmission intensity and exposure to vectors. In areas of seasonal malaria transmission, such immunological markers may complement entomological and parasitological surveillance. Here we analyzed IgG antibodies archived dried blood spot samples from 147 participants residing in a high malaria transmission area of western Kenya. The samples were collected at two time points: pre- and post-high malaria transmission season. IgG responses to *Anopheles gambiae* salivary peptide gSG6-P1 and three *P. falciparum* antigens (PfAMA1, PfCSP, PfMSP1) were quantified using a Luminex multiplex assay. Individual mosquito exposure was estimated from genotyping data using short tandem repeat (STR) markers, while concurrent infection data provided *P. falciparum* infection status. A paired t-test was used to assess seasonal changes, and Spearman's correlations evaluated associations between antibody levels and individual mosquito exposure. IgG responses to gSG6 and all parasite antigens increased significantly over the high malaria transmission season ($P<0.05$), particularly among children aged 5-15 years ($P<0.001$). However, seasonal antibody changes did not correlate with individual mosquito bite counts, highlighting that serological responses reflect broader seasonal exposure rather than discrete bite events. Notably, the response against PfAMA1 exhibited the strongest positive correlation with *P. falciparum* infection ($p<0.007$), while anti-PfMSP1 response showed a weaker pre-season association. Anti-gSG6 and anti-PfCSP IgG reactivity displayed positive but non-significant correlations, indicating limited predictive value for contemporaneous infection. These results demonstrate that IgG antibodies against blood-stage antigens, especially PfAMA1, provide a sensitive marker of recent malaria infection, while exposure-only markers are less informative at the individual level.

6705

HEMOGLOBIN GENOTYPE MODULATES HEMEINDUCED ENDOTHELIAL INFLAMMATION AT THE MALARIA-SICKLE CELL INTERFACE

Cecilia E. Botchway[1], Wesley Solomon[1], William Agbozo[1], Adriana Harbuzariu[2], Kwadwo A. Kusi[3], Jonathan K. Stiles[1]

[1]Morehouse School of Medicine, Atlanta, GA, United States, [2]Emory University, Atlanta, GA, United States, [3]University of Ghana, Accra, Ghana

Hemoglobin genotype influences vascular responses to hemolysis. Intravascular release of cell-free heme during malaria and sickle cell disease (SCD) triggers oxidative stress, endothelial damage, and inflammation. We generated patient-specific induced pluripotent stem cell-derived endothelial cells (iPSC-ECs) from individuals with HbAA, HbAS, and HbSS genotypes enrolled at Korle-Bu Teaching Hospital (Accra, Ghana) and profiled their responses to heme (38 μM). We also examined recombinant neuregulin-1 (NRG1) and cobalt protoporphyrin IX (CoPP; HO-1 inducer) as cytoprotective comparators. Outcomes included cell proliferation, junctional integrity, and inflammatory/angiogenic mediators (CXCL10, IL-6, IL-1β, TNF-α, Ang-2, VEGFA; ICAM-1/VCAM-1), along with clinical biomarkers (including plasma heme) linked to donor-matched iPSC-EC phenotypes; fetal hemoglobin (HbF) levels were quantified from dried blood spots. Heme decreased endothelial proliferation and increased inflammatory and adhesion markers across genotypes, with the largest median responses and variability among donors in HbSS iPSC-ECs. NRG1 consistently attenuated heme-induced inflammation, restored junctional integrity, and increased HO-1 expression across donors. Higher plasma heme levels in source participants correlated with stronger heme-triggered activation in paired iPSC-ECs, indicating the platform captures clinically relevant hemolysis burden. These findings demonstrate the utility of the iPSC-derived endothelial cell system as a non-invasive platform to model the effects of malaria and SCD-associated hemolysis on vascular pathophysiology. We are also able to assess the endothelial-targeted, cytoprotective effects of NRG1 and its modulatory effects on HO-1.

NRG1 showed robust cytoprotective effects, highlighting its potential as a therapeutic agent and supporting its use in biomarker-guided, genotype-specific strategies to reduce vascular inflammation associated with malaria and sickle cell disease.

6706

ANTIBODY LEVELS TO VARIANT AND CONSERVED *PLASMODIUM FALCIPARUM* ANTIGENS PREDICT REDUCTION IN PARASITE BURDEN IN MALIAN CHILDREN

Delesa Damena[1], Lauren Dang[1], Amadou Barry[2], Jonathan Renn[1], Santara Gaoussou[2], Almahamoudou Mahamar[2], Oumar Attaher[2], Djibrilla Issiaka[2], Robert Morrison[1], Santosh Misal[1], Alassane Dicko[2], Patrick Duffy[1], Michal Fried[1]

[1]National Institutes of Health, Bethesda, MD, United States, [2]Malaria Research and Training Center, University of Sciences, Techniques, and Technologies of Bamako, Bamako, Mali

Infected erythrocytes (IEs) are attractive targets for vaccine development because malaria-related pathology occurs at this stage of the parasite life cycle, and proteins expressed on IE surface are targeted by antibodies. We used immunoprecipitation followed by mass spectrometry (IP-MS) on plasma samples collected from susceptible and semi-immune children to identify targets of naturally acquired antibodies that may play a role in protection from malaria. We then investigated whether antibody levels to membrane-associated proteins identified by IP-MS, or *Plasmodium falciparum* erythrocyte protein 1(PfEMP1s) expressed in the IEs of young children, could predict a reduction in malaria disease risk. Significant reduction in risk of high parasite density infection was predicted by high antibody levels to DBLγ11 [OR (95%CI): 0.74 (0.63-0.86)] and DBLζ5 [0.80 (0.69-0.93)], as well as two *Plasmodium* helical interspersed subtelomeric proteins: PF3D7_0201600 [0.81 (0.70-0.94)] and PF3D7_0532300 [0.79 (0.68-0.92)]. Opsonic phagocytosis of beads coated with DBLγ11 and DBLζ5 was significantly higher in plasma samples from individuals in the top antibody level tertile than those in the lower tertiles ($p < 0.0001$). The relative phagocytosis index also correlated with antibody levels, with r^2 values of 0.61 ($p < 0.001$) and 0.49 ($p < 0.001$) for DBLγ11 and DBLζ5, respectively. The results suggest opsonic phagocytosis may mediate reduction in parasite density by antibodies to DBLγ11 and DBLζ5. Further studies are being conducted to characterize the candidate antigens identified in this study, and to explore the effector mechanisms that mediate their activities.

6707

EPITOPE-SPECIFIC ANTIBODY CROSS-REACTIVITY TO *PLASMODIUM FALCIPARUM* PROTEIN VARIANTS CORRELATES WITH AMINO ACID SEQUENCE CONSERVATION

Mary Mae Robinson[1], Scott Meredith[2], Patricia Ferrer[3], Jean Paul Courneya[1], B. Kim Lee Sim[4], Stephen L. Hoffman[4], Kim C. Williamson[2], Kirsten E. Lyke[1], Andrea A. Berry[1]

[1]Center for Vaccine Development and Global Health, University of Maryland School of Medicine, Baltimore, MD, United States, [2]Department of Microbiology and Immunology, Uniformed Services University School of Medicine, Bethesda, MD, United States, [3]Department of Microbiology and Immunology, Uniformed Services University of the Health Sciences, Bethesda, MD, United States, [4]Sanaria Inc., Rockville, MD, United States

Malaria due to *Plasmodium falciparum* (Pf) remains a leading cause of morbidity and mortality worldwide. Pf exhibits extensive genetic diversity, resulting in amino acid substitutions on surface-exposed regions of malaria proteins. Most previous studies have focused on the immune response to proteins from reference parasite strains (particularly the NF54/3D7 strain) without accounting for the protein variants present in circulating parasite strains. Understanding the extent to which antibody responses are cross-reactive versus strain-specific would inform next-generation vaccine development. In a series of controlled human malaria infections (CHMI) over two years, 8 participants were challenged with the Pf NF54 strain 3 or 4 times. IgG reactivity to 288 proteins inclusive of NF54 and up to 22 additional protein variants were measured before and after each CHMI on peptide microarrays, which contained overlapping linear peptides representing each protein's amino acid sequence. For each NF54 protein, regions with significant post-exposure reactivity were identified as immunodominant epitopes. We hypothesized that variants with greater amino acid similarity to NF54 would elicit comparable antibody binding. To test this, BLOSUM62 scores were used to estimate amino acid conservation between NF54 and each variant at immunodominant epitopes ($n = 690$). Antibody binding and variant:NF54 epitope amino acid sequence conservation were positively correlated ($\log_2 r = 0.28$), supporting the hypothesis that cross-reactivity is driven by sequence similarity. Although the array included only linear epitopes, we found that amino acids flanking the immunodominant epitopes were associated with measurable changes (both increases and decreases) in antibody reactivity. The degree of inter-variant conservation at immunodominant epitopes, the functional roles of these epitopes including involvement in host invasion, and the amino acid substitutions associated with retained cross-reactivity will be presented. These findings represent progress toward a predictive framework for antibody cross-reactivity to Pf antigens.

6708

SEROPREVALENCE OF ANTIBODIES TO PRE-ERYTHROCYTIC AND BLOOD-STAGE *PLAMODIUM FALCIPARUM* ANTIGENS AMONG ADULTS FROM MALARIA-ENDEMIC AND LOW-TRANSMISSION REGIONS OF INDIA

Ramya Madhavan, Poornima Saravanan, Lakshmi Raj, Chanduni Syed, Ajith Kumar, Akila Eswar, Oliver Esra, Ravish Sanghi Raju

Christian Medical College, Vellore, India

Malaria remains an important public health concern in India, with heterogeneous transmission across regions despite substantial progress in control efforts. Serological markers of exposure to *Plasmodium falciparum* can provide insights into cumulative malaria exposure, particularly in low-transmission settings where parasitological detection may underestimate prior infection. We aimed to assess seroprevalence of IgG antibodies to pre-erythrocytic and blood-stage antigens among adults from regions with differing malaria endemicity in India. Adults aged ≥18 years attending tertiary care departments were recruited after informed consent. Serum samples were collected and stored at –80°C. In-house ELISAs were standardised to quantify IgG responses against recombinant circumsporozoite protein (CSP)-based antigens and merozoite surface protein-1 (MSP-1), using the NIBSC malaria antibody standard (10/198). Seropositivity thresholds will be defined using samples from individuals originating from low-transmission regions. A total of 250 samples (125 from endemic and 125 from low-transmission regions) will be analysed. The ELISA platform for detection of antibodies has been successfully standardised. Sample collection is ongoing, with 150 participants recruited to date. Serological testing and comparative analysis across endemicity groups are in progress. This study will generate seroepidemiological data on *P. falciparum* exposure across diverse Indian populations and help identify individuals with prior malaria exposure for downstream immune profiling. These data will provide a critical foundation for defining naturally acquired antigen-specific immune signatures and prioritising *P. falciparum* epitopes relevant to multistage malaria vaccine development.

6709

INVESTIGATING HUMORAL IMMUNE RESPONSES TO IDENTIFY BROADLY REACTIVE ANTIBODIES TO *PLASMODIUM FALCIPARUM* INFECTED RED BLOOD CELLS

Courtney Tucker[1], Andrew Cooper[1], Cherrelle Dacon[1], Shanping Li[2], Kassoum Kayentao[3], Aissata Ongoiba[3], Boubacar Traore[3], Peter Crompton[2], Joshua Tan[1]

[1]Antibody Biology Unit, National Institutes of Health, Rockville, MD, United States, [2]Malaria Infection Biology and Immunity Section, National Institutes of Health, Rockville, MD, United States, [3]University of Sciences, Technique and Technology of Bamako, Bamako, Mali

Malaria remains a public health challenge with an estimated 282 million cases in 2024, a 3% (9 million) increase from the previous year. Groups most at risk for complications of malaria infection include women, children under 5, and the immunocompromised. The *Plasmodium* spp. life cycle is complex; as a result, there are many potential targets for the immune response. However, the clinical pathology associated with malaria infection is due in large part to the blood (asexual) stage. Building on previous work, our goal is to examine the antibody response to *Plasmodium*-infected red blood cell (iRBC) surface proteins using an antigen-agnostic approach. To this end, we will screen a polyclonal plasma from a Malian cohort with endemic exposure to *P. falciparum* to identify donors with reactivity to iRBCs. In addition, Malian donors will be screened for the ability to agglutinate infected red blood cells. In the next step, PBMCs will be sorted to isolate memory B cells (MBCs). We plan to use the Beacon optofluidics system to screen single MBCs for secretion of antibodies that bind to the iRBC surface, and have performed optimization experiments to validate this approach. With this optimization complete, we can proceed with high-throughput screening of MBCs for iRBC reactivity and ultimately generation of a panel of monoclonal antibodies that bind to the iRBC surface. We will identify the target antigens of these mAbs and test the mAbs for function against blood-stage *Plasmodium* parasites. Information gained from these experiments will provide further insight into the antigenic landscape on the surface of the iRBC and guide the development of future vaccines and therapeutics.

6710

PLASMODIUM VIVAX MEROZOITE SURFACE PROTEINS AS TARGETS OF ANTIBODY FC-MEDIATED FUNCTIONAL ANTIBODY RESPONSES

Kaitlin Pekin[1], Liriye Kurtovic[1], Gaoqian Feng[2], Daisy Mantila[3], Benishar Kombut[3], Maria Ome-Kaius[3], Moses Laman[3], Ivo Mueller[4], Leanne J. Robinson[1], Danny Wilson[5], D. Herbert Opi[1], James Beeson[1]

[1]Burnet Institute, Melbourne, Australia, [2]Nanjing Medical University, Nanjing, China, [3]Papua New Guinea Institute of Medical Research, Madang, Papua New Guinea, [4]Walter & Eliza Hall Inst, Parkville, Australia, [5]Adelaide University, Adelaide, Australia

Global malaria control has stalled over the past decade, with *Plasmodium vivax* emerging as of particular concern in endemic regions working towards elimination. Two malaria vaccines have been licensed, but do not target *P. vivax*, with few *P. vivax* candidates currently under development. A deeper understanding of immunity to *P. vivax* is required to inform vaccine development. Protective immunity to malaria is largely antibody-mediated, but the precise targets and mechanisms of action of immunity to *P. vivax* remain poorly understood. Merozoites, which infected red blood cells and are exposed to the immune system are ideal targets. Here, we evaluated merozoite surface proteins (MSPs) as targets of functional antibody responses to assist in identifying protective vaccine candidates. In a cohort of *P. vivax* exposed Papua New Guinean children, we evaluated the ability of antibodies to induce multiple protective functional responses to six *P. vivax* MSPs (MSP4, MSP5, MSP7 and MSP3). MSPs were targets of multiple functional antibody responses including Fcγ receptor (FcγRI, II and IIIa) binding and complement fixation with activity varying between antigens. We observed high FcγRI binding by antibodies to all antigens tested, which strongly correlated with IgG1 and IgG3. We observed high binding levels to FcγRII and FcγRIIIa but not across all antigens. We coated *P. vivax* MSPs to beads and used a monocytic cell line to quantify opsonic phagocytosis and measured antibody dependent cellular cytotoxicity (ADCC) using a FcγRIIIa reporter cell line. We observed high phagocytosis activity across all antigens that correlated with FcγRI binding, some antigens also correlated with FcγRII but not FcγRIIIa. Induction of ADCC varied between antigens and individuals; high responses were observed to half of the antigens. Our work provides new insights into *P. vivax* immunity, demonstrating that *P. vivax* merozoite surface proteins are major targets of potent antibody Fc-mediated effector functions including opsonic phagocytosis and ADCC. Targets and mechanisms of action identified here will support development of *P. vivax* vaccines and biomarkers of immunity.

6711

ASSOCIATION BETWEEN GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PD) DEFICIENCY AND MALARIA INFECTION AMONG PREGNANT WOMEN ATTENDING NSUTA HEALTH CENTRE

George Wireko Brobby Bonsu[1], Raymond Antwi[2], Jamila Ibrahim[1], Gloria Ama Asantewaa Oduro[1]

[1]University of Skills Training and Entrepreneurial Development, KUMASI, Ghana, [2]University of Education, Winneba, Mampong, Ghana

Sulphonamides are oxidant drugs that can trigger hemolysis in individuals with Glucose-6-Phosphate Dehydrogenase (G6PD) deficiency. Consequently, Ghana's anti-malaria drug policy recommends screening pregnant women for G6PD deficiency and excluding deficient individuals from Intermittent Preventive Treatment in pregnancy (IPTp) with sulphadoxine-pyrimethamine (SP). This study therefore assessed the seroprevalence of G6PD deficiency and malaria, and their association, among pregnant women attending Nsuta Health Centre. This cross-sectional study included 220 pregnant women at Nsuta Health Centre. About 2 mL of venous blood was collected per participant. G6PD activity was assessed using the methemoglobin reduction test. Giemsa-stained thick and thin smears were used for Plasmodium detection and speciation. Hemoglobin levels were measured with a Mindray BC-2800 analyzer to assess anemia. The overall prevalence of malaria was 15.4% (34/220), and the rate of positivity of G6PD deficiency was 13.2% (29/220). The most identified *Plasmodium* spp. was *Plasmodium falciparum* (12.7%; 28/220), followed by *Plasmodium malariae* (1.8%; 4/220), and the least was *Plasmodium ovale* (0.9%). Malaria and G6PD deficiency were prevalent among the age category 15-25 with 8.2% (18/220) and 5.9% (13/220), respectively. The overall seroprevalence for G6PD deficiency and malaria was 7.7% (17/220). In terms of education, the highest malaria prevalence, 6.8% (15/220), was observed in primary education holders. For occupation, the highest prevalence of 38.8% (26/254) of *Plasmodium infection* was recorded for traders.The prevalence and positivity rates of both malaria and G6PD deficiency were notably high among the study population. We therefore recommend routine and rigorous screening for G6PD deficiency during pregnancy, especially in malaria-endemic settings, to minimize the risk of hemolysis, anemia, and associated adverse pregnancy outcomes.

6712

AN ANTIGEN-AGNOSTIC APPROACH TO UNDERSTANDING ANTIBODY RESPONSES AGAINST BLOOD-STAGE *PLASMODIUM FALCIPARUM*

Mariah Lofgren[1], Andrew Cooper[2], Ababacar Diouf[3], Weizhi Zhong[4], Shanping Li[5], Safiatou Niare Doumbo[6], Didier Doumtabe[7], Jeff Skinner[8], Kassoum Kayentao[9], Aissata Ongoiba[10], Boubacar Traore[11], Carole A. Long[4], Peter Dobbs Crompton[12], Matthew Higgins[13], Joshua Tan[4]

[1]University of Oxford / National Institutes of Health, Rockville, MD, United States, [2]Rockville, MD, United States, [3]National Institute of Health, Rockville, MD, United States, [4]National Institutes of Health, Rockville, MD, United

States, [5]Medical Science & Computing, Rockville, MD, United States, [6]MRTC/DEAP/FMOS/USTTB, Bamako, Mali, [7]ICERMALI, Bamako, Mali, [8]NIH / NIAID / LIG, Rockville, MD, United States, [9]Malaria Research & Training Center, Bamako 1805, Mali, [10]Malaia Research and Training Center, Bamako, Mali, [11]Univ of Science Techniques & Tech of Bamako (USTTB), Bamako, Mali, [12]NIH NIAID, Rockville, MD, United States, [13]University of Oxford, Oxford, United Kingdom

The discovery of potent monoclonal antibodies (mAbs) against blood-stage *Plasmodium falciparum* merozoite antigens has elucidated mechanisms of red blood cell invasion and facilitated new clinical developments such as the development of the RH5 vaccine. Traditionally, mAb discovery approaches have focused on well-characterized antigens. However, more remains to be uncovered regarding the merozoite membrane antigenic landscape. Here, we used an antigen-agnostic approach to study the human antibody response to whole merozoites after natural malaria infection. Utilizing a cohort from Mali, a malaria endemic area that experiences seasonal transmission, we evaluated seroreactivity to *P. falciparum* merozoites. In order to focus on lesser-characterized antigens, we pre-blocked donor plasma with the immunodominant surface antigen MSP1 and subsequently tested plasma IgG binding to whole merozoites. Using this approach, we saw a wide range of reactivity post blocking with MSP1 and selected the top 15% of individuals with the highest antibody reactivity for downstream antibody discovery using the Beacon optofluidic system. This method has enabled the discovery of specific, high-binding anti-merozoite antibodies that have the potential to bind promising and uncharacterized targets on the parasite surface. Future work will involve assessing the potency of these antibodies using *in vitro* growth inhibition assays, as well as identifying the specific antigenic targets of these mAbs.

6713

ASSOCIATION BETWEEN HLA CLASS I AND II ALLELES AND RISK OF CLINICAL MALARIA WITHIN A MALIAN COHORT

Albert Park[1], Hyeseon Cho[1], Jeff Skinner[1], Youngsil Seo[1], Mathias Viard[2], Colm S. O'Huigin[2], Ludmila Krymskaya[3], Didier Doumtabe[4], Safiatou Doumbo[4], Aissata Ongoiba[4], Kassoum Kayentao[4], Boubacar Traore[4], Mary N. Carrington[2], Eric O. Long[3], Malcolm Sim[5], Peter D. Crompton[1]

[1]Malaria Infection Biology and Immunity Section, Laboratory of Immunogenetics, Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States, [2]Basic Science Program, Frederick National Laboratory for Cancer Research, National Cancer Institute, Frederick, MD, United States, [3]Laboratory of Immunogenetics, Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States, [4]Malaria Research and Training Centre, Mali International Center of Excellence in Research, University of Sciences, Techniques and Technologies of Bamako, Bamako, Mali, [5]Centre for Immuno-Oncology, Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom

Variations around the human leukocyte antigen (HLA) locus are emerging as an important contributor to malaria susceptibility. However, the association of specific HLA alleles with clinical outcomes is poorly understood. The HLA complex plays an essential role in activating the cell-mediated response, as they present processed antigens to T cells and act as ligands for killer immunoglobin-like receptors (KIRs) on NK cells. These components of the immune response play dynamic roles through parasite reduction and upregulation of inflammatory pathways that eventually drive pathological processes of malaria. We examined the association between HLA I and II alleles and the risk of symptomatic malaria in a cohort in Kalifabougou, Mali, where transmission is intense and seasonally predictable. We identified that two HLA I alleles, B*08:01 and B*18:01, were associated with protection and risk from clinical malaria, respectively. HLA II alleles DPA1*03:01:01 and DPB1*40:01:01 were also associated with an increased risk of clinical malaria. In parallel, we are analyzing whether these alleles influence parasitemia outcomes. As a step forward, we are phenotyping T cell effector responses from Malian peripheral mononuclear blood cells (PBMCs) exposed to *Plasmodium falciparum*-infected red blood cells (iRBCs) using flow cytometry. Our findings suggest that HLA class I and II alleles can differentially affect clinical malaria outcomes, further underscoring the importance of the cell-mediated response in *P. falciparum* immunity.

6714

PIPECOLIC ACID DRIVES NEUROLOGICAL DYSFUNCTION IN CEREBRAL MALARIA THROUGH A PUTATIVE GABAERGIC MECHANISM

Karina Garcia, Milany Bruno, Peter Bellotti, Simone Sidoli, Amanda Marley, Johanna P. Daily

Albert Einstein College of Medicine, Bronx, NY, United States

Cerebral malaria (CM) is a severe neurological complication of *Plasmodium falciparum* (*Pf*) infection characterized by coma and in survivors, a rapid, reversible loss of consciousness. To identify parasite-derived mediators of neurological dysfunction, we investigated pipecolic acid (PA), a lysine-derived metabolite with known neuromodulatory properties.PA levels were significantly elevated in plasma from children with CM compared to mild malaria. In an experimental cerebral malaria (ECM) model, brain PA levels correlated with the severity of neurological impairment, as measured by the Rapid Murine Coma and Behavioral Scale (RMCBS). To determine whether *Plasmodium* synthesizes PA via lysine degradation, we performed stable isotope tracing using ^{13}C-labeled lysine in both *Pb*A and *Pf*. These experiments demonstrated parasite-dependent PA production during the asexual blood-stages. To determine whether PA can induce a reversible coma, we administered PA via intracerebroventricular (ICV) injection in C57BL/6 mice, resulting in a rapid and reversible decrease in RMCBS. Based on prior evidence that PA modulates GABAergic signaling, we performed in silico modeling which predicted interactions between PA and GABA-associated receptors. We hypothesize that parasite-derived PA contributes to CM pathogenesis by inducing reversible coma through a GABAergic mechanism. Ongoing studies are testing whether pharmacologic blockade of GABA signaling via ICV administration can rescue PA-induced neurological dysfunction. Together, these findings support a model in which a parasite-derived metabolite crosses the blood-brain barrier and directly alters host neuronal signaling. This work establishes PA as a novel mediator of CM and highlights neurometabolic signaling as a potential therapeutic target for malaria-associated coma.

6715

DIFFERENTIALLY EXPRESSED *PLASMODIUM FALCIPARUM* RIFINS AND STEVORS IN SEVERE MALARIAL ANEMIA

Jonathan G. Lawton[1], Emily Stucke[2], Drissa Coulibaly[3], Sarika Kapadia[2], Savy Sebastian[2], Rafal Sobota[4], Jean-Paul Courneya[2], Bryan Cummings[2], Ankit Dwivedi[2], Antoine Dara[3], James Munro[2], Sakib Hussen[1], Abdoulaye Kassoum Koné[3], Karim Traoré[3], Bouréima Guindo[3], Bourama Tanagra[3], Amadou Niangaly[3], Youssouf Tolo[3], Mody Sissoko[3], Matthew Laurens[2], Amed Ouatarra[2], Bourema Kouriba[3], Ogobara Doumbo[3], Shannon Takala Harrison[2], Kirsten Lyke[2], Chris Plowe[2], Joana Silva[2], Mahamadou Thera[3], Mark Travassos[2]

[1]Goucher College, Baltimore, MD, United States, [2]University of Maryland School of Medicine, Baltimore, MD, United States, [3]University of Sciences, Techniques and Technologies, Bamako, Mali, [4]Northwestern University, Chicago, IL, United States

Severe malarial anemia (SMA) contributes substantially to pediatric malaria mortality in sub-Saharan Africa. Its pathogenesis is complex and incompletely understood, likely involving both host inflammatory dysregulation and parasite virulence mechanisms. During *Plasmodium falciparum* infection, parasites export variant surface antigens (VSA), including the highly diverse repetitive interspersed family (RIFIN) and subtelomeric variable open reading frames (STEVOR) multi-gene families. Although the contribution of RIFINs and STEVORs to SMA has not been defined, RIFIN subgroups bind human immune inhibitory and stimulatory receptors, suggesting capacity to modulate host innate inflammatory responses. We previously demonstrated enrichment of mRNA encoding

immune-targeting RIFINs in cerebral malaria (CM) compared with uncomplicated malaria (UM). Here, we hypothesized that parasites causing SMA express immune-modulating RIFINs and specific STEVOR subgroups at higher levels than those causing UM. Using RNA-sequencing and transcriptome assembly, we profiled *rif/stevor* gene expression in blood samples from Malian children aged 0-5 years with acute malaria (SMA, n=38; CM+SMA, n=12; UM, n=56) enrolled in a case-control study from 2014-2018. Transcripts were annotated using hidden Markov model-based classification, clustered across infections, and tested for cluster-level differential expression with linear models adjusted for parasitemia. Compared with UM, SMA infections featured two differentially expressed VSA clusters, one corresponding to an immune-modulating RIFIN and one to a strain-transcendent STEVOR. Comparing CM+SMA to UM, seven *rif* clusters were differentially expressed, including one corresponding to an immune-modulating RIFIN and one to a strain-transcendent RIFIN. These findings implicate *P. falciparum* VSAs in SMA pathogenesis via a biologically plausible immune-mediated mechanism. Next steps include identifying additional host receptors targeted by these VSAs using high-throughput AlphaFold3-based structural prediction and experimental validation, and comparing CM to SMA.

6716

A HUMAN INDUCED PLURIPOTENT STEM CELL (IPSC)-DERIVED NEUROVASCULAR UNIT MODEL REVEALS INFLAMMATORY AND METABOLIC DRIVERS OF NEURONAL INJURY IN CEREBRAL MALARIA

Alejandro Soto[1], Adnan Gopinadhan[2], Giselle Lima-Cooper[1], Meghana Imadabattini[1], Rylee Anderson[3], Jason Hughes[4], Kagan A. Mellencamp[1], Andrea Conroy[5], Thomas O'Connell[1], Chandy C. John[1], Scott Canfield[3], Dibyadyuti Datta[1]

[1]Indiana University School of Medicine, Indianapolis, IN, United States, [2]University of California San Francisco, San Francisco, CA, United States, [3]Indiana State University, Terre Haute, IN, United States, [4]University of Cincinnati, Cincinatti, OH, United States, [5]Indiana University, Indianapolis, IN, United States

The mechanisms linking blood–brain barrier (BBB) disruption to neurovascular unit (NVU) dysfunction and neuronal injury in cerebral malaria (CM) remain incompletely defined. *Plasmodium falciparum*-infected erythrocytes (IE) sequester on brain endothelial cells, yet how they induce neuronal injury without crossing the BBB is poorly understood. CSF studies in children with CM show inflammatory and injury-related changes associated with BBB disruption that are not fully reflected in plasma, highlighting a gap in how CNS-specific responses drive neuronal injury. Existing in vitro models have provided important insights into BBB dysfunction in CM; however, few systems investigate multicellular NVU injury in parallel with human disease markers. To address this, we developed an induced pluripotent stem cell (iPSC)-derived NVU tri-culture model with improved BBB integrity, comprising human brain microvascular endothelial cells, neurons, and astrocytes. ICAM-1/EPCR-binding HB3var03 IE strain co-culture induced rapid barrier disruption by 6 h, with reduced TEER, increased sodium fluorescein permeability, and delocalization of claudin-5, occludin, and ZO-1. Neuronal (tau, UCH-L1, NF-L) and glial (GFAP) injury markers were increased following exposure. Inflammatory profiling showed evidence of endothelial activation and NVU stress, while targeted metabolomics revealed compartment-specific disturbances, including elevated GABA, taurine, succinate, and β-alanine, consistent with redox imbalance and mitochondrial stress. Comparative metabolomic analyses identified overlapping signatures between the NVU model and CSF from children with CM, linking NVU-derived responses to human CNS metabolic alterations. Together, these findings demonstrate IE-mediated barrier disruption, inflammatory and metabolic signaling, and neuronal injury within the NVU, and provide a translational framework to investigate mechanisms of neural injury and CNS-targeted therapies in CM.

6717

BACTERIAL PRESENCE AND ITS POTENTIAL ROLE IN PEDIATRIC CEREBRAL MALARIA IN GHANA

Rhoda Yeboah[1], Winnefred Offih-Kyei[1], Elinam Adzo Agbobli[1], Caleb Danso-Coffie[1], Joanita Asirifi Yeboah[2], Andrews Osei Obese[3], Talent Akpoxornu Senanu[1], Ben Gyan[4], Gloria Ivy Mensah[1], Daniel Oduro[5]

[1]Noguchi Memorial Institute For Medical Research, Accra, Ghana, [2]Ghana, [3]LEGON, AA, Ghana, [4]Noguchi Memorial Inst for Med Res, Legon, Ghana, [5]UNIVERSITY OF GHANA, Accra, AA, Ghana

Cerebral malaria, a leading cause of pediatric mortality in Ghana, remains poorly understood beyond the role of *Plasmodium falciparum*. We investigated whether bacterial presence contributes to the severity of malaria disease in children. In a cross-sectional study of children aged 1-12 years with cerebral malaria, uncomplicated malaria, or no malaria, we collected blood, cerebrospinal fluid, and rectal swabs for bacterial culture, MALDI-TOF identification, and antimicrobial susceptibility testing. Among 52 isolates across 14 genera, rectal swabs exhibited the highest bacterial diversity. *Escherichia coli* prevalence was higher in children with cerebral malaria (63.6%) than in healthy controls (35.7%), suggesting a possible association, although the odds ratio (OR) was non-significant (OR=3.15, p=0.17). These isolates displayed multidrug resistance to cotrimoxazole (62.9%), ampicillin (51.9%), and tetracycline (51.9%), although 85% remained susceptible to ceftriaxone. While blood and cerebrospinal fluid cultures showed minimal growth, our findings suggest that bacterial presence may influence the pathogenesis of cerebral malaria. These results may support integrated strategies for empiric antibiotic management and warrant further investigation into host-microbiome interactions in severe pediatric malaria.

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CARDIAC FINDINGS IN SEVERE MALARIA ASSESSED BY SERIAL POINT-OF-CARE ULTRASOUND

Mutia Kehwalla[1], Patrick Tshilab[2], Agnes M. Mtaja[3], Jean-Bertin Bukasa Kabuya[4], Matthew M. Ippolito[1]

[1]Johns Hopkins Malaria Research Institute, Bloomberg School of Public Health, Baltimore, MD, United States, [2]Saint Paul's Mission Hospital, Nchelenge, Zambia, [3]National Heart Hospital, Lusaka, Zambia, [4]National Health Research and Training Institute, Ndola, Zambia

Severe malaria (SM) remains a major cause of morbidity and mortality among children in endemic regions, yet the effects of malaria on the heart are poorly understood. We investigated longitudinal changes in echocardiographic findings in Zambian children with SM enrolled in a prospective cohort study in Nchelenge District, Zambia. Participants underwent serial point-of-care ultrasound (POCUS) examinations at admission, discharge, and 3 months post-discharge. Measurements included ejection fraction (EF), fractional shortening (FS) from parasternal long- and short-axis views, and pericardial effusion (presence/absence). Pulmonary B-lines were also assessed. Longitudinal changes were assessed using linear mixed-effects models, paired t-tests, and McNemar's test. Among 117 enrolled children, no significant changes were observed in EF or FS across admission, discharge, and follow-up. However, a high prevalence of pericardial effusion during acute malaria was observed, typically resolving by the 3-month follow-up visit (56% vs. 10%, paired *t* test $p < 0.001$). Participants with B-lines on lung POCUS had twice the odds of pericardial effusion at baseline compared to those without (OR = 2.37, 95% CI: 1.0-5.7, p = 0.04). Younger age was associated with higher odds of pericardial effusion and higher EF and FS values. Malnutrition was significantly associated with reduced EF. Children with pericardial effusion during hospitalization had higher FS at baseline, potentially reflecting inflammatory hyperkinesis or measurement artifacts. SM in children was associated with transient pericardial effusion in this cohort. The higher observed prevalence of B-lines in children presenting with pericardial effusion suggests that SM may be associated with third-space fluid

accumulation driven by systemic inflammation. Although acute malaria was not associated with reduced cardiac function, malnutrition and younger age were associated with lower EF.

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BIOMARKER PATHWAYS OF COGNITIVE IMPAIRMENT IN UGANDAN CHILDREN WITH CEREBRAL MALARIA AND SEVERE MALARIAL ANEMIA: A MULTI-GROUP STRUCTURAL EQUATION MODELING ANALYSIS

Caitlin Bond[1], Dibyadyuti Datta[1], Paul Bangirana[2], Ruth Namazzi[2], Robert Opika Opoka[3], Giselle Lima-Cooper[1], Andrea Conroy[4], Rashelle Musci[5], Matthew Michael Ippolito[6], Chandy C. John[7]

[1]Indiana University School of Medicine, Indianapolis, IN, United States, [2]Makerere University, Kampala, Uganda, [3]College of Health Sciences, Makerere Univ, Kampala, Uganda, [4]Indiana University, Indianapolis, IN, United States, [5]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [6]Johns Hopkins University School of Medicine, Baltimore, MD, United States, [7]Indiana University School of Medicine, Saint Paul, MN, United States

Children surviving cerebral malaria (CM) and severe malarial anemia (SMA) face high risk of long-term cognitive impairment, but pathways linking biomarkers of systemic and brain injury to outcomes remain poorly understood. We analyzed data from 429 Ugandan children under 5 years with microscopy-confirmed *Plasmodium falciparum* CM (n=169) or SMA (n=260) enrolled in two prospective cohort studies. Univariable associations between 65 blood biomarkers and 12-month cognitive outcomes (age-adjusted Mullen Scales of Early Learning z-scores) were assessed using adjusted regression and elastic net regression. In parallel, biomarkers were incorporated into a multi-group structural equation model (SEM) with partial metric invariance and full information maximum likelihood to simultaneously test shared and disease-specific pathways in CM and SMA. Univariable analyses revealed different predictor profiles by disease group: neurologic biomarkers GFAP (glial fibrillary acidic protein) and tau were among the strongest biomarker predictors of worse cognition in CM, while in SMA nutritional and sociodemographic factors were selected by elastic net. In the SEM, inflammatory, endothelial, and uric acid markers did not independently predict cognitive outcomes. Both groups shared a renal-neuronal-astroglial pathway: hemolysis predicted renal impairment, which predicted neuronal injury, which predicted astroglial activation (all $p<0.001$). Astroglial injury predicted cognitive impairment in CM ($\beta=-0.34$, $p=0.001$), but not SMA ($\beta=-0.12$, $p=0.09$), and supported mediation of the neuronal injury-cognition association. The indirect pathway from renal dysfunction to cognition via neuronal and astroglial injury was significant in CM ($p=0.006$) but not SMA. A tau×GFAP interaction amplified impairment in CM ($\beta=-0.33$, $p<0.001$). Together, univariable regression and SEM analyses indicate astroglial activation is a CM-specific mediator of cognitive impairment. In SMA, cognitive outcomes may reflect mechanisms beyond acute brain injury biomarkers, suggesting distinct pathways across severe malaria phenotypes.

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KINETICS OF TISSUE PLASMINOGEN ACTIVATOR AND PLASMINOGEN ACTIVATOR INHIBITOR 1 IN *PLAMODIUM VIVAX* MALARIA.

Miriam E. Cantero[1], Luis Stevens Ramos G.[2], Yira S. Reyes[2], Rosa A. Oviedo[3], Natalia P. Echeverri[3], Daniel J. Mestra[4], Carlos J. Castro[5], Maria F. Yasnot[5]

[1]University of Córdoba, Chemistry Department, Monteria - Cordoba - Colombia, Colombia, [2]Bacteriologist, University of Córdoba, Bacteriology Department, Montería - Córdoba, Colombia, [3]Student of Bacteriology, Montería - Córdoba, Colombia, [4]Epidemiologist at San Jerónimo Hospital in Montería, Montería - Córdoba, Colombia, [5]University of Córdoba, Bacteriology Department, Montería - Córdoba, Colombia

Malaria infection remains one of the leading causes of morbidity and mortality worldwide, and malaria caused by *Plamodium vivax* induces significant alterations in hemostatic balance, yet the temporal dynamics of fibrinolytic markers are not yet well understood. The objective of this study was to evaluate the kinetics of tissue plasminogen activator (tPA) and plasminogen activator inhibitor-1 (PAI-1) over a 72-hour period in 72 patients with *P. vivax* malaria confirmed by diagnostic PCR, hospitalized at E.S.E Hospital San Jerónimo de Montería, and 15 healthy volunteers. Plasma concentrations of tPA and PAI-1 were quantified using molecule-specific ELISA, and longitudinal variations were analyzed over three consecutive days. On day 1, plasma concentrations of tPA (7540.8 ± 3267.1 pg/mL) and PAI-1 (142946.7 ± 43842.2 pg/mL) were significantly elevated compared to the healthy control group (tPA = 2346.1 ± 613.4 pg/mL and PAI-1 = 106291.1 ± 30285.9 pg/mL), indicating acute endothelial activation accompanied by a predominant hypofibrinolytic state driven by the increase in PAI-1. By day 2, both biomarkers showed a significant decrease, reflecting a partial modulation of the fibrinolytic response. By day 3, tPA levels approached those of the control group, while PAI-1 demonstrated a more pronounced reduction, falling below baseline concentrations, suggesting a progressive restoration of fibrinolytic balance. Statistical analysis confirmed significant differences between the different follow-up points for both markers ($p < 0.05$), indicating a dynamic and reversible alteration of the fibrinolytic system. The observed pattern supports a model in which malaria triggers an initial compensatory but insufficient fibrinolytic activation, overcome by inhibitory mechanisms that favor microthrombotic processes. The subsequent decrease in PAI-1 may reflect the therapeutic response and the resolution of inflammation. These findings highlight the relevance of fibrinolytic biomarkers as potential indicators of disease progression and endothelial recovery in malaria.

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PARASITE VARIANT SURFACE ANTIGEN EXPRESSION IN SEVERE MALARIA IN ZAMBIAN CHILDREN

Emily M. Stucke[1], Jonathan G. Lawton[2], Jean-Paul Courneya[1], Sarika P. Kapadia[1], Savy M. Sebastian[1], Jean-Bertin Bukasa Kabuya[3], William J. Moss[4], Joana C. Silva[5], Matthew M. Ippolito[4], Mark A. Travassos[6]

[1]University of Maryland School of Medicine, Baltimore, MD, United States, [2]Goucher College, Baltimore, MD, United States, [3]Tropical Diseases Research Centre, Ndola, 8, Zambia, [4]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [5]Univ of Maryland School of Medicine, Silver Spring, MD, United States, [6]University of Maryland, Baltimore, MD, United States

Severe malaria kills hundreds of thousands of children across sub-Saharan Africa annually, accounting for most malaria-related deaths globally. We have not yet identified the exact parasite genes responsible for severe disease that could be targets for vaccine or drug development. Parasite variant surface antigens – genetically diverse proteins expressed on the surface of infected erythrocytes – appear to play a critical role in severe malaria pathogenesis. These include *Plasmodium falciparum* erythrocyte membrane protein-1 (PfEMP1), RIFIN, and STEVOR proteins encoded by the *var*, *rif*, and *stevor* multigene families. PfEMP1 antigens mediate parasite sequestration via adherence to host endothelial receptors. The functions of RIFINs and STEVORs are less well understood, but include binding to erythrocyte and immune cell receptors. Our central hypothesis is that severe malarial disease is primarily the result of a subset of virulent parasite variant surface antigens that are common across sub-Saharan Africa, raising the possibility of severe malaria interventions effective across disparate regions. We built a novel informatics pipeline to sequence and *de novo* assemble variant surface antigen transcripts from whole blood RNA-seq with a custom capture array to enrich for variant surface antigen detection. Using this approach, we identified subsets of PfEMP1s, RIFINs, and STEVORs associated with cerebral malaria and severe malarial anemia in Mali. We will sequence variant surface antigen transcripts in severe malarial anemia and cerebral malaria cases and matched uncomplicated malaria controls in Zambian children enrolled in a longitudinal cohort study in holoendemic Nchelenge District, Luapula Province. We will determine whether the variant surface antigens associated with severe malaria in Zambian children are

similar to those previously identified in West African children. Our results have implications for the potential of universal vaccines and treatments for severe malaria across sub-Saharan Africa.

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BLOOD GROUP ANTIGEN QUANTITATION ON THE SURFACE OF PLATELETS AND RED BLOOD CELLS DIFFER BETWEEN ASYMPTOMATIC AND CLINICAL MALARIA

Katherine McGraw[1], Priscilla Suleman-Johnstone[2], Nicole Montello-Pierce[1], Monica Soko[2], Patricia Mawindo Mariana[2], Iset Medina Vera[3], Kami Kim[3], Karl Seydel[1]

[1]Michigan State University, East Lansing, MI, United States, [2]Blantyre Malaria Project, Kamuzu University of Health Sciences, Blantyre, Malawi, [3]University of South Florida, Tampa, FL, United States

The ABO(H) blood group system, which is widely recognized in transfusion medicine, has been implicated in malaria pathogenesis. ABO(H) antigens are found at high levels on the surface of red blood cells (RBCs) and in variable amounts on other cells such as platelets (PLTs). The ABO(H) system is composed of carbohydrate antigens A, B, and H, with the H antigen acting as the precursor to A and B antigens. In blood group O, H antigen remains unmodified, whereas in A and B blood groups the majority is modified by the addition of A or B. The role of the ABO(H) system in susceptibility to malaria has been studied with blood group O showing relative protection from cerebral malaria. At the other end of the *P. falciparum* infection disease spectrum is asymptomatic (ASX) malaria, a sub-clinical infection that results in no clinical symptoms. We aimed to investigate whether the quantities of ABO(H) antigens on PLTs and RBCs play any role in susceptibility to these infections. PLTs and RBCs were isolated from whole blood samples collected from Malawian pediatric patients with ASX (n=77) or uncomplicated malaria (UCM) (n=81), and surface ABO(H) expression was quantified with lectins via flow cytometry. When evaluating ABO(H) antigen expression on platelets, we found that the overall H antigen levels were significantly increased on PLTs from children with ASX when compared to PLTs from children with UCM ($p<0.001$). Furthermore, when comparing H antigen expression between children with ASX and UCM within each ABO blood group, ASX PLTs from groups A, B, and O showed significantly higher levels ($p=0.01$, $p=0.03$, and $p=<0.001$, respectively) compared to UCM. RBCs also showed a significant difference in H antigen expression in groups A and O, with ASX being higher ($p=<0.01$ and $p=0.01$, respectively) when compared to UCM. These findings suggest that H antigen expression is variable between cell types and disease states that may influence host-parasite interactions and contribute to differences in progression to clinical malaria and disease severity. Identifying the mechanisms by which this occurs could provide a valuable insight into malaria pathogenesis.

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MALARIA ASSOCIATED HEMOLYSIS INDUCES PATHOGENIC TRANSCRIPTIONAL REPROGRAMMING IN HUMAN MICROGLIA

Wesley Solomon[1], William Agbozo[1], Cecilia Botchway[1], Adriana Harbuzariu[2], Jonathan K. Stiles[1]

[1]Morehouse School of Medicine, Atlanta, GA, United States, [2]Emory University, Atlanta, GA, United States

Severe malaria is frequently accompanied by intravascular hemolysis, generating cell free hemoglobin and free heme that amplify oxidative stress, vascular dysfunction, and neuroinflammation. In cerebral malaria, these processes contribute to blood-brain barrier disruption and neurocognitive sequelae, yet the mechanisms linking hemolysis to central nervous system injury remain poorly defined. Microglia are the resident immune cells of the central nervous system that support surveillance and tissue repair, but sustained activation can promote inflammatory injury. We hypothesized that disease relevant free heme reprograms human iPSC derived microglia toward a pathogenic pro inflammatory state consistent with cerebral malaria and that adjunctive therapies may attenuate these responses. Human iPSC derived microglia were exposed to free heme (38μM). Transcriptional responses were assessed using pathway level transcriptomics integrated with multiplex secretome profiling and cell viability assessment. Therapeutic modulation was evaluated using cobalt protoporphyrin (CoPP, 5μM), atorvastatin (ATV, 2μM), or neuregulin 1 (NRG1, 100ng/ml). Free heme induced transcriptional reprogramming characterized by activation of tissue stress, oxidative stress, and IL 1 family signaling, with induction of HMOX1 and inflammatory mediators including IL1β and CXCL8, and suppression of homeostatic microglial identity programs. Secretome profiling demonstrated increased IL1β, IL6, IL8, MCP1, and VEGF, consistent with blood-brain barrier disruption. Heme reduced microglial viability to approximately 52% of control. Therapeutic effects were agent specific: CoPP enhanced stress tolerance programs, ATV restored homeostatic transcriptional signatures, and NRG1 improved survival while amplifying inflammatory signaling. These findings demonstrate that malaria associated hemolysis drives a pathogenic microglial phenotype linking free heme exposure to neuroinflammation and blood-brain barrier relevant signaling and establish a tractable human microglia model for evaluating adjunctive neuroprotective strategies.

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ACHIEVING COVERAGE PARITY IN SEASONAL MALARIA CHEMOPREVENTION AMONG NOMADIC AND SETTLED POPULATIONS: A MULTI-STATE EQUITY ANALYSIS IN FIVE NIGERIAN STATES

Jennifer Ugochi Chukwumerije[1], Ridwan Jega[1], Olabisi Adebo Ogunmola[2], Daniel Chukwuyere Emeto[3], Ayodeji Joseph Balogun[4], Benjamin Amao[1], Abdulrazaq Abdullahi[1], Usman Husseini[1], Chibuzo Oguoma[1]

[1]Malaria Consortium, Abuja, Nigeria, [2]Malaria Consortium, FCT, Nigeria, [3]Abuja, FC, Nigeria, [4]Ogbomosho, OY, Nigeria

Nomadic and mobile pastoralist populations in Nigeria face structural risks of exclusion from campaign-based health interventions due to seasonal mobility and administrative invisibility. Although Nigeria implements the world's largest Seasonal Malaria Chemoprevention (SMC) programme, evidence on equity of reach across mobility strata remains limited. We compared SMC coverage and sulfadoxine-pyrimethamine plus amodiaquine (SPAQ) adherence between nomadic/mobile and settled children across five Nigerian states. A cross-sectional multistate analysis of the 2025 SMC campaign was conducted in Kebbi, Sokoto, Bauchi, Nasarawa, and Oyo, where nomadic populations ranged from 5% to 26% of the target population. Independent End-of-Cycle Lot Quality Assurance Sampling (LQAS) surveys provided subgroup-specific coverage and adherence estimates. Absolute differences, relative coverage ratios, and 95% confidence intervals were calculated, with pooled estimates derived from aggregated subgroup counts. Among 6,229,963 children aged 3-59 months targeted, 10.8% were classified as nomadic/mobile. Coverage among nomadic/mobile children ranged from 93.5% to 96.7% compared with 92.1% to 97.5% among settled children. The pooled absolute difference was 1.2 percentage points (95% CI: 0.7-1.7), with no consistent mobility-associated disadvantage. Adherence, defined as caregiver-reported completion of Day 2 and Day 3 SPAQ doses, exceeded 94% in both groups. Qualitative findings highlighted adaptive microplanning, cross-LGA coordination, culturally familiar distributors, and intensified supervision as key inclusion strategies. Despite variation in nomadic population density, SMC achieved high coverage and adherence with minimal differences between groups. Structured microplanning and adaptive delivery strategies may mitigate mobility-associated exclusion in large-scale malaria prevention programmes.

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SUPPORTING EVIDENCE-BASED DECISION-MAKING IN AN ERA OF CONSTRAINED FUNDING: MAPPING THE SEASONAL MALARIA CHEMOPREVENTION EVIDENCE LANDSCAPE

Anna Y. Burkhard[1], Richard Kajubi[2], Asadu Sserwanga[1], Angharad Steele[1], Bronwen G. Davies[1], Shannon Shisler[3], Cem Yavuz[4], Anil Thota[5], Olusola Oresanya[6], Chukwudi Arnest Nnaji[1], Jane Achan[2]

[1]Malaria Consortium, London, United Kingdom, [2]Malaria Consortium, Kampala, Uganda, [3]International Initiative for Impact Evaluation, Washington DC, DC, United States, [4]International Initiative for Impact Evaluation, London, United Kingdom, [5]International Initiative for Impact Evaluation, Toronto, ON, Canada, [6]Malaria Consortium, Abuja, FC, Nigeria

Seasonal malaria chemoprevention (SMC) is a key intervention for reducing malaria morbidity and mortality among children in areas with highly seasonal transmission. Systematically assessing the evidence landscape and identifying knowledge gaps is essential to maximise research impact, guide future studies, and inform policy and programme decisions. This is particularly important as current global funding constraints emphasise the need for prioritisation and efficient use of available resources to address existing research gaps. To address this, Malaria Consortium are developing an Evidence Gap Map (EGM) to systematically catalogue and visualise the current evidence base for SMC across epidemiological, programmatic, and contextual domains. In partnership with the International Initiative for Impact Evaluation (3ie), we constructed an a priori intervention-outcome framework informed by consultations with technical experts and programme implementers, and further validated by the Chemoprevention Alliance. The framework covers areas such as intervention design; delivery models; coverage and adherence; drug resistance; cost and cost-effectiveness; scalability; and equity considerations. We will search academic databases and grey-literature repositories for studies, screening using a two-stage process with independent reviewers. Included studies will be coded using a structured taxonomy capturing study design, populations, outcomes, and implementation characteristics. The evidence will be reported narratively and presented through an interactive, publicly accessible map to support decision makers in navigating the evidence landscape. The EGM will identify priority evidence gaps to inform policy, guide investment in research, and support evidence-informed optimisation of SMC programmes in eligible settings.

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EFFECT OF HOUSEHOLD FOLLOW-UP ON ITN UTILIZATION IN BURKINA FASO: A COMMUNITY-BASED IMPLEMENTATION PROCESS

Issa Ouedraogo[1], Ousmane Badolo[1], Yacouba Ouedraogo[1], Yacouba Belem[1], Heather Harison[2], Gladys Tetteh[2], Habi Ouattara[3]

[1]JHPIEGO Corporation, Ouagadougou, Burkina Faso, [2]JHPIEGO Corporation, Baltimore, MD, United States, [3]SP/Palu, Ouagadougou, Burkina Faso

Malaria remains the leading cause of outpatient consultations, hospitalizations, and mortality in Burkina Faso. To address this burden, the country relies on several strategies, including the use of insecticide-treated nets (ITNs). In the Integrated Health Services (IHS) project regions (Nando, Nakambé and Djôrô) ITNs coverage is high (87% among children under one year and 96% among pregnant women), yet utilization remains below 70%, falling short of the national target of 85%. In these regions, only 65% of the population has knowledge about malaria prevention using ITN.In response, the IHS project strengthened household-level monitoring of ITNs use which is a routine intervention conducted by facilitators using the tools set up by the ministry of health. to foster community outreach efforts. Focused on pregnant women and children under five, each community facilitator, covering 45 households in three villages per month, verifies actual ITNs use the night before the visit, checks the quality of ITN installation, and sensitize families on malaria prevention measures. They also, refer eligible pregnant women and children under one year old who have not received routine ITNs to health facilities. The use of ITN is verified through declarations made by household members. The targeted villages were selected based on key malaria indicators from the health facilities to which they are affiliated. Facilitators were trained in the implementation of this approach and in the use of digital data collection tools. From June to September 2025, the 90 facilitators visited 8,457 households, reaching 1, 819 pregnant women and 8,493 children under five. The results indicate that 90.13% of ITNs were properly installed, and that 7,974 people received malaria prevention messages.The high proportion of correctly installed ITN and the large number of individuals reached with prevention messages highlight the added value of community facilitators in bridging the gap between ITN coverage and effective use. Its effectiveness could be strengthened further by allowing facilitators to conduct revisits to verify the sustainability of the behaviors adopted.

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INDOOR RESIDUAL SPRAYING HAS PROVEN TO BE AN EFFECTIVE AND OPERATIONALLY FEASIBLE INTERVENTION THAT REDUCES MALARIA TRANSMISSION BY TARGETING INDOOR-RESTING *ANOPHELES* MOSQUITOES IN RWANDA

Epaphrodite Habanabakize

Rwanda Biomedical Centre, Kigali, Rwanda

Malaria continues to pose a major public health challenge in Rwanda, particularly in high-transmission districts such as Gisagara. Indoor Residual Spraying (IRS) is a key vector control intervention that reduces malaria transmission by targeting indoor-resting *Anopheles* mosquitoes. This study evaluated the impact of IRS on malaria incidence in Gisagara District during the October 2025-February 2026 implementation period, aiming to inform malaria control and elimination strategies.A quasi-experimental pre- and post-intervention study design was applied using routinely collected malaria surveillance data from health facilities. Confirmed malaria cases were analyzed as incidence per 1,000 population, and test positivity was used as a proxy indicator of transmission intensity. Malaria indicators were compared for four months before and four months after IRS implementation, with additional comparison between IRS-targeted and non-targeted sectors to assess attributable impact.Following IRS implementation, malaria incidence in targeted sectors declined from 176 to 11 cases per 1,000 population, representing a 94 % reduction. Test positivity rate decreased from 14.2% to 8.6%, indicating a significant reduction in transmission intensity ($p < 0.05$). Non-targeted sectors experienced smaller reductions, further supporting the effectiveness of IRS. High spray coverage (>99%), appropriate timing during peak transmission seasons, strong community acceptance, and effective coordination across health system levels were identified as key determinants of success.The study demonstrates that IRS substantially reduced malaria incidence and transmission intensity in Gisagara District within a short implementation period. Despite limitations related to routine data use, short follow-up duration, and potential confounding factors, IRS proved to be an effective and operationally feasible intervention. These findings support continued prioritization and potential scale-up of IRS as part of Rwanda's malaria elimination framework.

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EVALUATING THE IMPACT, ACCEPTABILITY AND COMMUNITY EXPERIENCES OF SEASONAL MALARIA CHEMOPREVENTION. A MIXED-METHOD STUDY IN KAKUMA AND KALOBEYEI REFUGEE CAMPS IN KENYA

Emmah C. Kimachas[1], Beatrice Rotich[1], Catherine Kafu[1], George Angolio Ambani[1], Rebecca Lokwang[1], Lucy Achieng Abel[1], Caroline Ngina[1], Victor Osiare[2], Elizabeth Ayoma[2], Dennis Opondo[3], Ronald Rop[3], Felix Kibet[4], Danson Barasa[4], David Kipngetich[3], Sila Monthe[4], Damaris Wanjiku[5], Jesse Wambugu[5], Edwin Mbugua[2], David Ekai[6], Avi Kenny[7], Diana Menya[8], Wendy P. O'Meara[7]

[1]AMPATH, Eldoret, Kenya, [2]Catholic Relief Services, Lodwar, Kenya, [3]Kenya Redcross, Kakuma, Kenya, [4]International Rescue Committee, Kakuma, Kenya, [5]UNHCR, KAKUMA, Kenya, [6]Department of Health Services and Sanitation, Turkana County, Turkana, Kenya, [7]Duke University, Durham, NC, United States, [8]Moi University, Eldoret, Kenya

Kakuma and Kalobeyei refugee camps in Turkana, Kenya is home to approximately 302,000 refugees. The camp experience recurrent malaria outbreaks, particularly among children. In 2025, Seasonal Malaria Chemoprevention (SMC) was implemented for the first time across four cycles to reduce malaria burden during peak transmission. We conducted a mixed-methods evaluation using routine facility testing electronic records from seven health facilities (~800,000 records cumulatively) and eight focus group discussions with 96 caregivers. Quantitative analysis applied a difference-in-discontinuities approach for cases in 2025 to estimate cases averted at the age eligibility cutoff (5 years), while qualitative data explored acceptability, barriers to uptake as well as their experiences with SMC The quantitative results demonstrated a high disease burden in the refugee camps. Following SMC implementation, seasonal peaks in 2025 were lower compared to 2024. A significant shift in malaria patterns at age 5 was observed (p=0.018), indicating intervention impact. An estimated 3,114 cases (95% CI: 604-5,624) were averted among children under five. Qualitative findings highlighted strong community acceptability driven by high trust in Community Health Promoters (CHPs), the perceived severity of malaria and strong sensitization efforts. Caregivers reported reduced illness among children during SMC. However, barriers included concerns over safety on an when SMC is taken on empty stomach due to food shortages, and skepticism regarding the exclusion of the native host population. SMC substantially reduced malaria burden and was highly acceptable in this refugee setting. Strengthening community engagement, improving sensitization, and addressing delivery gaps and integrating nutritional gaps could further enhance impact.

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OPTIMIZING MASS CAMPAIGNS THROUGH THE INTEGRATION OF HOUSEHOLD ENUMERATION AND DOORTODOOR DISTRIBUTION OF LONGLASTING INSECTICIDAL NETS (LLINS): A PILOT EXPERIENCE IN FRIA, GUINEA

Fatoumata Battouly Diallo[1], Ibrahima Diallo[1], Abdourahamane Diallo[2], Charles Agossa Lawson[1], Ibrahima Kalil Keita[2], Fakouma Camara[1], Alioune Camara[2], Aissatou bobo Bah[1], Chrestien Yameni[3], Suzanne Van hulle[4]

[1]Catholic Relief Services, Conakry, Guinea, [2]National Malaria Control Program, Conakry, Guinea, [3]Catholic Relief Services, Dakar, Senegal, [4]Catholic Relief Services, Baltimore, Guinea

Since 2013, Guinea has conducted four national mass distribution campaigns of long-lasting insecticidal nets (LLINs), cumulatively distributing 30,915,125 LLINs. Challenges related to the availability and accessibility of LLINs in remote, mining, and rural areas have led to adaptations in operational strategies. In 2025, the National Malaria Control Program (NMCP) initiated an innovative pilot strategy in the Fria health district, Boké region, combining household enumeration with door-to-door distribution. This approach aimed to improve population access to LLINs, ensure 100% distribution to enumerated households, and strengthen the achievement of universal coverage within the framework of the campaign. Two modalities were implemented: (i) in urban areas, door-to-door enumeration followed by distribution at fixed sites starting the following day; and (ii) in rural areas, integrated door-to-door enumeration and distribution conducted on the same day. The entire process was digitalized using tablets, data management, card information, and stock management with CommCare, as well as LLIN scanning using QR codes. The implementation of this strategy took place over six days, from June 28 to July 3, 2025. Out of a total of 87,215 LLINs planned, 84,500 were made available and 64,723 were distributed, representing a distribution rate of 77%, with coverage of 94% of enumerated households (26,934 households). The LLIN scanning rate reached an average of 95%, demonstrating the feasibility of this technological innovation. Despite logistical challenges related to the rainy season and transportation in hard-to-reach areas, the pilot approach showed strong potential power to improve completeness, data quality, and efficiency for future campaigns. The results support a gradual scale-up of the enumeration-distribution linkage and the systematic scanning of LLINs during future national campaigns.

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THE RATIONALE FOR AND LONG JOURNEY TO AN OPTIMAL CRYOVIAL FOR CRYOPRESERVED EUKARYOTIC CELLS LIKE PFSPZ VACCINES AND PFSPZ CHALLENGE.

Eric R. James, Dimitri Koutzoumis, Diana Perez, Lixin Gao, Minglin Li, Yonas Abebe, Sumana Chakravarty, Jeremy Guth, Tooba Murshedkar, David Dolberg, Thomas Laurence Richie, B. Kim Lee Sim, Stephen L. Hoffman

Sanaria Inc, Rockville, MD, United States

Plasmodium falciparum sporozoites (PfSPZ), the active ingredient in PfSPZ vaccines and PfSPZ Challenge, being eukaryotic organisms, must be stabilized by cryopreservation, stored and distributed below -150 °C in liquid nitrogen vapor phase (LNVP). Using off-the-shelf-screwcap cryovials, an effective cryopreservation protocol was developed, initially by testing a range of cryoprotectant additive compounds, their concentrations, incubation times and temperatures, and cooling/warming rates for *P. yoelii* SPZ, with ID50s and protection against challenge as readouts. The method was adapted to PfSPZ with membrane integrity and hepatocyte potency assays as readouts. In clinical trials where PfSPZ Challenge was used, ID100s with ~10.5-11.5 days to blood smear positivity have been consistent at 3,200 PfSPZ/volunteer for different manufactured lots and for different storage durations. However, screw cap vials are not acceptable for Phase 3 and a unique ~1 mL vaccine cryovial with a septum had to be developed. The process began in the UK/Japan with company #1, then company #2 in Alabama and now company #3 in Michigan. Fill-finish scale up involved developing high throughput (HT) automated filling, capping/sealing and HT cryopreservation based on the SBS 96-vial/box standard for automated liquid handling. This format permits high density (HD) storage and HD shipping/distribution which reduces logistics costs. Standard screwcap cryovials do not provide container closure integrity (CCI) below ~-80 °C because the cap and vial materials contract at different rates with cooling, allowing gas ingress and egress at cryogenic temperatures. Additionally, the cryovial must have an integral septum to maintain product sterility during syringe preparation in rural and remote locations. Until now, no such vial has existed commercially that incorporates all the features of HT fill finish, optimum cooling and warming rates, HT cryopreservation, HD storage, HD distribution and integrated sterile protected septum. The Sanaria Cryovial meets all CCI requirements and is a critical component of the PfSPZ vaccines and Challenge manufacturing program.

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ESTABLISHING BASELINES FOR IMPLEMENTING MALARIA ELIMINATION AND CONTROL INTERVENTIONS ON ANNOBÓN ISLAND, EQUATORIAL GUINEA

Restituto Mba Nguema Avue[1], David Galick[1], Jeremias Nzamio Mba Eyono[1], Nestor Rivas Bela[1], Crisantos Nsue Abeso[1], Marcos Mbulito Iyanga[1], Santiago Eneme Mbang[1], Matilde Riloha Rivas[2], Jordan Smith[3], Wonder Philip Phiri[1], Carlos Alberto Guerra[4], Guillermo Alberto Garcia[3]

[1]MCD Global Health, Malabo, Equatorial Guinea, [2]National Malaria Control Program, Malabo, Equatorial Guinea, [3]MCD Global Health, Hallowell, ME, United States, [4]MCD Global Health, Quito, Ecuador

Annobon Island, the smallest and most remote territory of Equatorial Guinea, has remained outside the scope of organized malaria control. In 2025, the Government of Equatorial Guinea adopted Vision 2030, a national elimination strategy that extends malaria control and elimination activities beyond Bioko Island to the entire country, including Annobon. Planning effective interventions on Annobon requires reliable population denominators and baseline malaria prevalence data, neither of which existed. The Bioko Island Malaria Elimination Project (BIMEP) used its household mapping infrastructure, combining its spatial decision support system, the Campaign Information Management System (CIMS), with open source building polygons, to conduct a household census on Annobon Island in late 2025. A team of five technicians completed the mapping in three weeks, identifying 2,131 households of which 928 are inhabited, with a resident population of 2,912 individuals, establishing for the first time reliable denominators for planning and monitoring malaria interventions on the island. A malaria indicator survey (MIS) will be conducted in May 2026 to establish baseline prevalence and key malaria indicators. By the time results are presented, both the household baseline and the first prevalence estimates for Annobon will be available, providing the foundation needed to plan, implement, and measure the impact of malaria elimination activities under Vision 2030. BIMEP's mapping methodology demonstrates that establishing baseline denominators is operationally feasible in any setting where population data are absent, using existing infrastructure and a small field team.

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COMMUNITY AT THE HELM: SOCIAL BEHAVIOR CHANGE INNOVATIONS ACCELERATING MALARIA PREVENTION AND CARE IN BONNY ISLAND, NIGERIA

Anastasia Isodje, Adebusola Oyeyemi, Adetomiwa Gbolade

NLNG, Port Harcourt, Nigeria

Malaria remains a major public health challenge in Nigeria's coastal and riverine communities, where environmental conditions, population mobility, and socio-cultural norms sustain transmission. Bonny Island reflects this reality: awareness of malaria is high, yet preventive and care-seeking behaviors remain inconsistent. The Nigeria LNG-funded Bonny Malaria Elimination Project (BNYMEP) responds to this gap through a community-driven Social and Behavior Change (SBC) model. BNYMEP adopted Human-Centered Design to co-create interventions with community volunteers, ward focal persons, faith and traditional leaders, youth groups, and schools. This led to locally owned, behavior-shaping activities integrated into existing social networks and institutions e.g. peer-led dialogues, SBC integration into church and women's group meetings, school health clubs, community events, strengthened linkages to health facilities and vector control services. Early learning shows that trusted local actors are central to transforming "malaria awareness" into concrete household practices. The abstract shares the BNYMEP model, key behavior insights, HCD co-creation process, implementation experience, and emerging results. It also reflects on challenges, adaptations, and implications for scaling within national malaria programs. Comparison of baseline and midline data shows clear behavioral improvements across Bonny Island. LLIN utilization increased significantly from 54.3% to 75.6%. Early treatment-seeking behavior rose from 6.9% percent to 31.1%, demonstrating improved urgency in testing and care-seeking. School absenteeism due to malaria dropped from 10.6% to 3.7%, suggesting reduced disease burden among children. Qualitative findings from community dialogues further revealed stronger collective ownership of malaria control, with residents increasingly linking consistent prevention and prompt treatment to family wellbeing, productivity, and community development. Together, these results show meaningful shifts driven by locally led SBC interventions.

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FROM DATA TO DECISIONS: PREPARING ANALYSIS-READY SURVEILLANCE DATA FOR SUBNATIONAL MALARIA TAILORING IN TANZANIA

Yasini Kokoda

Ifakara Health Institute, Dar Es Salaam, United Republic of Tanzania

Availability of electronic health information systems such as District Health Information Software 2 (DHIS2) and the Master Facility List (MFL) has expanded the use of routine surveillance data for malaria modelling to support decision-making. However, these data are not originally designed for analytical or modelling purposes, requiring extensive pre-processing to produce reliable and analysis-ready inputs. Despite increasing utilization of routine data for subnational tailoring (SNT) and risk stratification, standardized and reproducible data processing workflows remain poorly documented. This study outlines the procedures used to prepare surveillance data for SNT in Tanzania, while highlighting key practical considerations. Facility level malaria testing indicators (2017-2024), disaggregated by age (<5 and ≥5 years), were extracted from DHIS2, standardized, and linked to the MFL using health facility identifiers. Council names were harmonized to align DHIS2 data with the shapefile. Facilities that never reported malaria indicators during the study period were excluded. Because DHIS2 does not distinguish missing values from zeros, missing values in low-risk strata were assumed to be zeros, while those in moderate- and high-risk strata were treated as true missing. Records with confirmed cases exceeding tests and extreme outliers identified using the interquartile range method were also set to missing. All missing values were subsequently filled using imputation methods. Important observations included mismatches in facility and council information across DHIS2, MFL, and shapefiles, leading to missing facility attributes, as well as anomalies such as a facility reporting ~120,000 tests in a single month compared to typical values in the hundreds. Key lessons emphasize the need for multi-system synchronization, layered data quality checks, contextual understanding, and cross-sector coordination. This framework provides a transparent, reproducible approach for preparing routine malaria data for downstream analysis and decision-making.

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FASTTRACKING MULTIPLE FIRSTLINE THERAPIES (MFT) MONITORING: INTEGRATING ACTDISAGGREGATED INDICATORS INTO DHIS2 TO OPERATIONALIZE MFTS IN BURKINA FASO

Wendnso Maria Sidonie Gouem[1], Moctar Sankara[2], Aissata Barry[3], Yacouba Belem[1]

[1]Jhpiego, Ouagadougou, Burkina Faso, [2]MoH, Ouagadougou, Burkina Faso, [3]SP/PALU, Ouagadougou, Burkina Faso

Burkina Faso adopted Multiple FirstLine Therapies following evidence of declining artemetherlumefantrine efficacy. However, routine systems lacked ACTdisaggregated data to monitor implementation. Integrating ACTspecific indicators into DHIS2 addresses this gap, enabling improved MFT monitoring, ACT forecasting, and evidencebased malaria case management. Under the STOPAMDR project, MFTspecific indicators were integrated into Burkina Faso's National Health Information System (DHIS2/EndosBcombined F) ahead of the planned national revision of data collection tools in late 2026. Led by SP/Palu in collaboration with key stakeholders such as DSSE/MoH, district data managers, and malaria stakeholders, the process included institutional advocacy, co-design and

validation of indicators, DHIS2 configuration, and data simulations to ensure quality and program ownership. The workshop resulted in the validation of MFT transition indicators, updates to facility reporting tools to capture ACT-specific data, configuration of indicators in Endos BF, and data simulation to ensure reporting quality. Key deliverables included a facility data collection sheet, a digital reporting form, dashboard indicators, and a data entry guide. Future actions focus on training data managers and facility staff and rolling out the revised tools across all five MFT regions. The process highlighted the importance of strong institutional leadership, multisectoral collaboration, participatory indicator design, and sustained technical support for effective ACT monitoring in DHIS2. Proactive integration of MFT indicators into Burkina Faso's National Health Information System shows that ACTdisaggregated reporting can be implemented through a focused, institutionally led process without waiting for a full system overhaul. This enables realtime monitoring of AL, DHAPPQ, and ASPY at the facility level; strengthens supply chain quantification and policy decisionmaking; and offers a practical, replicable model for other DHIS2based MFT implementations.

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CONCURRENT ENTOMOLOGICAL AND MALARIA INDICATOR SURVEY SAMPLING ON BIOKO ISLAND, EQUATORIAL GUINEA: LINKING VECTOR DENSITY AND HOUSEHOLD TRANSMISSION RISK

Nestor Rivas Bela[1], Gedeon Yohannes[1], Prudencio Bibang Engono Efiri[1], Marcelino Nsue Nguema Mbang[1], David Galick[1], Liyu Teklemichael[2], Wonder Philip Phiri[1], Peter Billingsley[2], Carlos A. Guerra[3], Guillermo A. Garcia[2]

[1]MCD Global Health, Malabo, Equatorial Guinea, [2]MCD Global Health, Hallowell, ME, United States, [3]MCD Global Health, Quito, Ecuador

Understanding the spatial relationship between vector density and malaria transmission is critical for targeting vector control, yet entomological data are rarely collected alongside malaria prevalence surveys in a way that enables direct household-level linkage. During the 2025 malaria indicator survey (MIS) on Bioko Island, Equatorial Guinea, the Bioko Island Malaria Elimination Project (BIMEP) conducted concurrent entomological collections across 24 primary sampling units (PSUs) selected based on *Plasmodium falciparum* prevalence and housing quality characteristics associated with mosquito entry. PSUs were prioritized to span a range of transmission intensity, with 20 representing the highest prevalence and 4 serving as lower-prevalence comparators. A composite housing quality score derived from window screening, eave type, and wall construction was used to prioritize PSUs with a higher proportion of houses susceptible to mosquito entry. Light trap catches were deployed in 20 houses per PSU on alternating nights over 8 weeks, timed to coincide with the MIS to enable direct linkage of entomological and epidemiological data at the household level. A total of 459 light trap collection nights were completed across 230 households, 230 indoors and 229 outdoors. Anopheles were caught in only 18% of houses, with a mean capture of 5 and a maximum of 40 individuals per house. Although households in light trap-positive houses showed higher *Pf* prevalence than light trap-negative houses (18.3% versus 17.2%), this difference was not statistically significant. Additional analyses will be presented of the association between vector density, housing characteristics, time since IRS application, and household *Pf* positivity, which are all integrated through a spatial decision support system. These preliminary findings confirm the operational feasibility of concurrent MIS and entomological sampling to establish a linked dataset positioned to answer the question of whether household-level vector density predicts malaria transmission risk independently of housing quality and IRS exposure and address programmatic decision making.

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ADAPTIVE ENTOMOLOGICAL SURVEILLANCE ON BIOKO ISLAND, EQUATORIAL GUINEA: A SAMPLING FRAMEWORK FOR CHARACTERIZING VECTOR BEHAVIOR AND TRANSMISSION RISK

Gedeon Yohannes[1], Nestor Rivas Bela[1], Prudencio Bibang Engono Efiri[1], Mathias Leonard[1], Heliodoro Noha[1], Juan Edu Bang[1], Marcelino Nsue Nguema Mbang[1], David Galick[1], Wonder Philip Phiri[1], Peter F. Billingsley[2], Carlos Alberto Guerra[3], Guillermo A. Garcia[2]

[1]MCD Global Health, Malabo, Equatorial Guinea, [2]MCD Global Health, Hallowell, ME, United States, [3]MCD Global Health, Quito, Ecuador

Entomological surveillance is essential for guiding vector control decisions in malaria control and elimination settings, yet many programs rely on static sentinel site designs that limit spatial representativeness and introduce sampling bias. In 2026, the Bioko Island Malaria Elimination Project transitioned from a fixed sentinel surveillance approach to an adaptive sampling framework to generate more representative data on vector abundance, behavior, and transmission potential on Bioko. Six sentinel sites were defined based on malaria prevalence and geographic distribution. Collections are conducted twice monthly on alternating weeks. Houses are selected from a defined pool of eligible structures with open eaves and no window screening, with systematic rotation to minimize repetition. Adult mosquitoes are collected indoors and outdoors using human landing catches (HLC), light trap catches, and Prokopack aspirations. Field supervisors perform overnight morphological identification and dissections to assess parity as a proxy for transmission potential. Data presented are preliminary from February and March 2026 and pertain exclusively to HLC. A total of 2,712 *Anopheles* were collected in 51 houses across the six sites. Of these, 62.6% (n=1698) were collected outdoors versus 37.4% (n=1014) indoors, indicating a strong exophagic feeding pattern. Collections were spatially heterogeneous, with 81% of mosquitoes (n=2206) caught in 22% of the houses (n=11). The overall parity rate was 0.76, suggesting a high proportion of the vector population has survived long enough to potentially transmit malaria. Collections were achieved across all six sites with minimal house repetition, confirming operational feasibility. The high proportion of outdoor biting, spatial heterogeneity, and elevated parity rate underscore the need for spatially targeted vector control strategies that extend beyond indoor-focused interventions. The systematic rotation of collection houses and consistent temporal coverage make this an efficient, sustainable framework for entomological monitoring across malaria control and elimination settings.

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DEVELOPING GLOBAL PUBLIC GOODS FOR MALARIA SURVEILLANCE: LESSONS LEARNED FROM CREATING ACTWATCH LITE - A STANDARDIZED PRIVATE SECTOR ASSESSMENT TOOLKIT

Katelyn Woolheater[1], Keith Esch[1], Manfred Accrombessi[2], Erica Wang[1], Paul Bouanchaud[1]

[1]PSI, Washington, DC, United States, [2]PSI, Contonou, Benin

Effective malaria control and elimination require reliable data beyond robust routine surveillance. To generate data for these gaps, global health initiatives emphasize the development of open-access, standardized toolkits that serve as global goods, allowing countries and partners to generate comparable evidence while avoiding fragmented, project-specific assessments. One such toolkit has been developed to measure private sector malaria markets, enabling consistent measurement of availability, price, and market share as well as case management practices and supply chain characteristics across diverse settings. The ACTwatch Lite toolkit, developed by PSI with funding from the Gates Foundation, translates a complex research methodology into a structured set of adaptable components, including a core indicator framework, survey tools, sampling guidance, and analysis templates. The toolkit is designed for adaption and use by national malaria programs and implementing partners. The toolkit

was piloted in Benin, Cameroon, and Nigeria in 2023-2024, then reviewed and validated by a WHO-convened panel of experts. Several lessons emerged while developing this global good. First, a well-defined indicator framework is essential to ensure data gaps are captured and comparable across settings. Second, modular toolkit components allow for easier adaptation to different epidemiological contexts, regulatory environments, outlet types, and program priorities. Third, sustained engagement with stakeholders throughout the design and development process supports feasibility, relevance, and importantly, uptake of the resulting product. Clear documentation and publicly accessible tools are critical to enabling reuse and adaptation of assessment methodologies. The development of standardized, open-access tools is an important approach to strengthen sustainable malaria surveillance and evidence generation. Lessons from this experience highlight practical considerations for translating complex research methods into scalable global goods that support program decision-making and improve data availability.

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ESTIMATING *PLASMODIUM* SPECIES-SPECIFIC SEROPREVALENCE FROM MULTIPLEX SEROLOGIC DATA USING MULTI-PATHOGEN MODEL OF CROSS-REACTIVITY

Madiha Shafquat[1], Megan O'Driscoll[2], Sophie Bérubé[3], Andrea C. Carcelen[1], Celso Monjane[4], William J. Moss[1], Sonia Hegde[1], Saki Takahashi[1]

[1]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [2]Hopitaux universitaires de Geneve, Genève, Switzerland, [3]University of Florida, Gainesville, FL, United States, [4]Instituto Nacional de Saúde, Maputo, Mozambique

Estimating pathogen-specific seroprevalence is challenging in settings where multiple antigenically similar pathogens co-circulate and can elicit cross-reactive antibody responses. In malaria, sympatric *Plasmodium* species share conserved genes that encode antigenically similar proteins which can elicit cross-reactive antibody responses, complicating the measurement of species specific antibody levels. We applied MultiSero, a multivariate mixture model, to jointly estimate species-specific seroprevalence and between-species antibody cross-reactivity from responses to merozoite surface protein-1_{19} (MSP1-19) measured using a multiplexed serological assay. Data were drawn from a previously published cross-sectional, population-based serosurvey in Zambezia Province, Mozambique, including 1,292 individuals with multiplex bead assay measurements targeting MSP1-19 from *P. falciparum*, *P. malariae*, *P. ovale*, and *P. vivax*. Estimated prevalence was highest for *P. falciparum* (0.84, 95% CrI: 0.81–0.87), with lower estimates for *P. malariae* (0.16, 95% CrI: 0.13–0.19) and *P. ovale* (0.09, 95% CrI: 0.07–0.12). *P. vivax,* known to be absent in this setting, was fixed at a seroprevalence of 0. Cross-reactivity estimates indicated that *P. falciparum* exposure contributes disproportionately to antibody responses across species, suggesting that naïve analyses may overestimate non-falciparum exposure. In contrast, cross-reactive contributions from *P. malariae* and *P. ovale* appeared more limited and asymmetric, implying that these antibody responses are less likely to drive signal across other antigens. These findings highlight the importance of cross-reactivity in estimating species-specific exposure using serologic data. Cross-reactivity estimates also provide evidence of asymmetric, cross-reactive humoral immune responses to *Plasmodium* species. Ongoing work extends this analysis to additional settings, to evaluate generalizability across transmission contexts with various circulating *Plasmodium* species.

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FIELD-DEPLOYABLE TAQMAN QPCR PLATFORM FOR DETECTING MOLECULAR MARKERS FOR MALARIA SURVEILLANCE

Madhavinadha Prasad Kona[1], Armel Tedjou[2], Francesco Buongiorno[3], Lorenzo Colombo[4], Charles S. Wondji[5], George Dimopoulos[6]

[1]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [2]Centre for Research in Infectious Diseases (CRID), Yaoundé, Cameroon, [3]Hyris Research Center, Viale Lancetti, Milano, Italy, [4]Hyris Srl, Milano, Italy, [5]Liverpool Sch of Tropical Medicine, Liverpool, United Kingdom, [6]Johns Hopkins University Bloomberg School, Baltimore, MD, United States

Effective malaria control strategies require rapid, decentralized molecular diagnostics that provide timely, site-level surveillance data to inform program decision-making. We developed and validated a field-ready bCUBE qPCR platform for comprehensive malaria vector surveillance capable of detecting Anopheles species, *Plasmodium* infections and species, blood-meal sources, and insecticide resistance markers. The system integrates a one-step sample preparation buffer enabling simple homogenization workflows for individual or pooled mosquitoes, eliminating the need for complex DNA extraction protocols in resource-limited settings. Multiplex TaqMan qPCR assays were designed and validated for the bCUBE 3-channel platform to simultaneously detect multiple surveillance markers. Mixed multiplex triplex assays combine marker types, including parasite targets, vector species targets, blood-meal host targets, or insecticide resistance markers, enabling a single bCUBE TaqMan qPCR run to answer multiple surveillance questions simultaneously. These customizable triplex assays can be tailored to the epidemiology and program needs of each study area, maximizing relevance, efficiency, and cost-effectiveness. Field validation in Cameroon demonstrated successful on-site deployment with complete workflows completed within four to five hours. The platform's automated interpretation via mobile application enhances usability for health workers generating surveillance data for National Malaria Control Programs. This portable, user-friendly molecular surveillance system enables decentralized, real-time monitoring of critical malaria transmission indicators supporting evidence-based vector control interventions in endemic regions.

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LEVERAGING NATIONAL SUPERCOMPUTING INFRASTRUCTURE FOR SCALABLE PREDICTION OF *PLAMODIUM FALCIPARUM* - HOST PROTEIN INTERACTIONS

JP Courneya[1], Jonathan Lawton[2], Emily Stucke[3], Savy M. Sebastian[1], Alexander John Laurenson[2], Shannon Takala Harrison[4], Mark A. Travassos[5]

[1]University of Maryland Baltimore, Baltimore, MD, United States, [2]Baltimore, MD, United States, [3]University of Maryland School of Medicine, Baltimore, MD, United States, [4]University of Maryland School of Medicine, Churchville, MD, United States, [5]University of Maryland, Baltimore, MD, United States

Severe malaria remains a leading cause of childhood mortality worldwide, yet the molecular interactions between parasite proteins and human host receptors that drive pathogenesis are incompletely understood. Large-scale *in silico* protein interaction screening using tools such as AlphaFold2 can help prioritize targets for experimental validation, but the computational demands often exceed institutional resources. We developed a bioinformatics pipeline deployed on national supercomputing infrastructure through an NSF ACCESS allocation on ACES (TAMU-HPRC) to perform high-throughput screening of *Plasmodium falciparum* proteins against a curated panel of 102 human host targets. Parasite sequences from polymorphic antigen families, including PfEMP1s and RIFINs, were derived from field isolate mRNA collected during acute malaria infections in children, along with publicly available reference data. *In silico* translated sequences generated using getORF were screened against candidate human receptors using AlphaPulldown with an AlphaFold2 multimer backend, followed by pDockQ interface quality scoring. The pipeline separated CPU-intensive feature generation from GPU-accelerated inference on NVIDIA H100 nodes and was orchestrated using Slurm batch scheduling with array jobs and checkpointing. As a proof of concept, we generated 2,822 parasite antigen structure predictions and over 300 parasite-human complex models, each scored for interaction quality. This large-scale computing approach enabled high-throughput screening not feasible on institutional hardware and produced structure-informed interaction candidates prioritized for experimental validation, generating translational preliminary data relevant to investigation of severe malaria mechanisms and vaccine development.

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EVIDENCE-BASED APPROACH TO IMPROVING AVAILABILITY AND QUALITY OF SEVERE MALARIA DATA IN WESTERN KENYA

James Andati[1], Elvis Oyugi[1], Dan Abaja[1], Beatrice Kemunto Machini[2], Donald Apat[1]

[1]PATH, Nairobi, Kenya, [2]NMCP, KNH Nairobi, Kenya

Standardized disease coding and reporting are essential for generating high-quality data to guide malaria service delivery. Routine Kenya Health Information System (KHIS) data reviews in high-burden counties of Western Kenya indicate sub-optimal (<80%) reporting of severe malaria data from admitting facilities, currently at 63%, due to gaps in documentation of clinical management, limited capacity in ICD-11 coding, and discordance in reporting across inpatient registers, monthly summary tools, and KHIS. This evaluation assessed the effect of peer mentorship on reporting completeness and data concordance. We analyzed inpatient KHIS data and purposively selected 166/956 (17.5% of all admitting facilities) with <80% reporting rates. In July and November 2025, expert coders mentored 221 health records officers (HRIOs). They reviewed inpatient registers, cross checked and validated data entries against the monthly summary tool and KHIS. Action plans were developed to address reporting gaps, including backlogs, with follow-up visits to facilities not meeting targets to resolve emerging challenges. These facilities reported 11,311 severe malaria cases (16.9% of admissions), with a case fatality rate of 0.9% (101/11,311) and proportionate mortality of 5.1% (101/1,974). In June 2025, 76.5% (169/221) of HRIOs lacked ICD-11 training. Reporting rates improved from 53.9% in June to 89.7% in December 2025. Data concordance between monthly summary tools and KHIS increased from 43.3% to 49.3% with variation between counties (17.0%-68.7%) partly reflecting differences in clearance of facility level reporting backlogs. Concordance was higher for morbidity (54.1%) than mortality data (44.3%). Peer mentorship substantially improved reporting completeness and strengthened HRIO capacity in ICD-11 coding. Limited improvement in concordance likely reflects persistent challenges including retrospective data entry backlogs, varying levels of HRIO capacity and misalignment between source documents and reporting tools. We recommend continued mentorship to sustain the gains, improve severe malaria data availability and quality.

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TRENDS OF MALARIA SLIDE POSITIVITY RATES IN SENTINEL SITES ACROSS GHANA, 2022-2025

Benjamin Kwaku Abuaku[1], George Adu Asumah[2], Collins Stephen Ahorlu[1], Alexander Asamoah[2], Eunice Obeng Amoako[1], Paul Boateng[2], Addo Christabel[1], Nana Yaw Peprah[2], Nancy Odurowah Duah-Quashie[1], Mohammed Wahjib[2], Neils Ben Quashie[1], Kwadwo Ansah Koram[1], Paul Hilarius Abiwu[2]

[1]Epidemiology Department, Noguchi Memorial Institute for Medical Research, College of Health Sciences, University of Ghana, Accra, Ghana, [2]National Malaria Elimination Programme, Ghana Health Service, Accra, Ghana

As part of efforts to monitor the impact of malaria interventions in Ghana, 30 representative health facilities were selected as sentinel sites by the National Malaria Elimination Programme (NMEP) in collaboration with the Noguchi Memorial Institute for Medical Research (NMIMR) to generate data for monitoring malaria slide positivity rates (mSPRs) across the country. This surveillance activity involves the storage of malaria slides for every third suspected malaria case within each health facility. The slides are examined by WHO-certified microscopists. Analysis of data from the 30 sentinel sites showed a significant decline in the overall malaria slide positivity rate (mSPR) from 11.6% (95% CI: 11.4 - 11.9) in 2022 to 6.9% (95% CI: 6.7 - 7.1) in 2025 (p=0.000). A sensitivity analysis using health facilities that were able to store at least one-month of malaria slides within each quarter of 2022-2025 was also done. Analysis of microscopy results from 22 eligible health facilities similarly showed a significant decline in the overall malaria slide positivity rate (mSPR) from 11.8% (95% CI: 11.5 - 12.1) in 2022 to 7.3% (95% CI: 7.1 - 7.6) in 2025 (p=0.000). Facility-specific results showed significant decline of mSPR in 16 of the 22 health facilities. We conclude that interventions deployed over the years have resulted in significant gains in Ghana.

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UPTAKE OF MALARIA VACCINATION AMONG CHILDREN AGED 5-15 MONTHS: ENHANCED SEASONAL MALARIA VACCINATION IN KEBBI STATE, NIGERIA

Michael Ekholuenetale[1], Oluwafunmilayo Ibikunle[1], Attahiru Muhammed Bello[1], Kolawole Maxwell[1], Nasiru Umar[2], Chuks Nnaji[3], Katherine Theiss-Nyland[4], Olusola Oresanya[1]

[1]Malaria Consortium, Abuja, Nigeria, [2]Malaria Programme, Kebbi State Ministry of Health, Nigeria, Kebbi, Nigeria, [3]Malaria Consortium, London, United Kingdom, [4]London School of Hygiene and Tropical Medicine, U. of London, London, United Kingdom

Nigeria accounts for the highest global malaria burden. At the subnational level, Kebbi State reports among the highest prevalence of malaria. The Enhanced Seasonal Malaria Vaccination (ENHANCE) study was implemented in Kebbi State to increase malaria vaccine coverage among young children in two Local Government Areas (Birnin Kebbi and Koko/Besse). The study aimed to generate real-world evidence on optimizing malaria vaccine coverage in highly seasonal transmission settings. In 2025, a hybrid malaria vaccine delivery model (combining seasonal age-based model with community demand creation and health information to build on routine age-based delivery) was assessed using a three-arm, non-randomized cluster-controlled study design. Baseline and endline evaluations were analysed using difference-in-differences (DiD) to estimate malaria vaccine uptake. At baseline, first dose coverage varied across LGAs (34.0-75.6%). At endline, first dose coverage increased to 89.0-97.9%. The DiD estimation showed significant intervention effects. Coverage increased by 14.1 percentage points more in Birnin Kebbi than in the control LGA, Sakaba, which implemented routine age-based vaccination only. Koko/Besse had a 2.4 percentage point higher but non-significant improvement than Sakaba. Access to health information was strongly associated with vaccination, with individuals having information access showing twice the odds of vaccination (aOR = 2.12, 95% CI: 1.15 - 3.89; p = 0.015). The findings suggest that the hybrid model improved vaccine uptake compared to routine age-based delivery. A hybrid seasonal-age-based delivery, combined with community demand creation and health information campaigns, showed improved malaria vaccine coverage and supports considerations for scale-up in settings with highly seasonal malaria transmission.

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DATA VISUALIZATION TO INFORM MALARIA VACCINE PROGRAM DECISIONS: VIEW-HUB AS A TOOL FOR ADVOCATES AND POLICYMAKERS

Marley Jurgensmeyer, **Anurima Baidya**, Victoria Willens, Karoline Walter, William J. Moss

International Vaccine Access Center, Johns Hopkins Bloomberg School of Public health, Baltimore, MD, United States

Malaria was responsible for 282 million cases and 610,000 deaths across 80 countries in 2024. The recent introduction of malaria vaccines offers new opportunities to mitigate these health impacts, particularly among children living in malaria-endemic regions. Monitoring malaria burden and vaccine introduction can help decision-makers optimize limited resources to protect children against malaria. The open-access platform VIEW-hub (view-hub.org) tracks global malaria vaccine introduction, use, and disease burden. Interactive maps and tables display data on vaccine introduction, delivery approach, product, and timing of doses. Data are regularly updated to permit near real-time reporting. Data from the World Health Organization on malaria cases and deaths are also visualized. As of March 2026, five countries have introduced malaria vaccines nationally and 20 countries have introduced subnationally. Four countries are planning to introduce malaria vaccines. Of countries that have introduced, all are Gavi-eligible and

located in the African region. Most countries (23, or 92%) use an age-based approach to deliver vaccines, while the remaining two countries (Guinea-Bissau and Mali) use a hybrid approach. Fourteen (56%) countries use the R21/MatrixM vaccine, ten (40%) countries use the RTS,S/AS01 vaccine, and one country (Ghana) uses both vaccines. Of the 47 countries with the highest disease burden (measured as the top quartile of confirmed malaria cases in 2024), about half (25, or 53%) have introduced malaria vaccines nationally or subnationally. The remaining countries with high disease burden are settings where vaccines could have a significant impact and where efforts to introduce malaria vaccines should be focused. VIEW-hub is a useful resource for tracking global malaria vaccine introduction and implementation decisions. As global partners commit financing and technical support for malaria vaccines, timely data can help countries prioritize support, monitor progress, and ensure that future malaria vaccine introductions have the greatest potential impact on disease.

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EVALUATING THE GENETIC DIVERSITY OF THE *PLAMODIUM VIVAX* SIAP2 LOCUS: A PROMISING CANDIDATE FOR AN EFFECTIVE MALARIA VACCINE?

Carlos J. Castro-Cavadía[1], Sergio E. Plata-Pineda[2], Laura X. Cárdenas-Munévar[2], Sindy P. Buitrago[2], Diego Garzón-Ospina[3]

[1]Universidad de Córdoba, Monteria, Colombia, [2]Universidad Pedagógica y Tecnológica de Colombia, Tunja, Colombia, [3]Universidad del Cauca, Popayán, Colombia

Malaria is the deadliest parasitic disease in the world. Traditional control measures have become less effective; hence, there is a need to explore alternative strategies, such as antimalarial vaccines. However, designing an anti- *Plamodium vivax* vaccine is considered a challenge due to the complex parasite biology and the antigens' high genetic diversity. Recently, the sporozoite invasion-associated protein 2 (SIAP2) has been suggested as a po- tential antigen to be considered in vaccine design due to its significance during hepatocyte invasion. However, its use may be limited by the incomplete understanding of gene/protein diversity. Here, the genetic diversity of pvsiap2 using *P. vivax* DNA samples from Colombia was assessed. Through PCR amplification and sequencing, we compared the Colombian sequences with available worldwide sequences, revealing that pvsiap2 displays low genetic diversity. Molecular evolutionary analyses showed that pvsiap2 appears to be influenced by directional selection. Moreover, the haplotypes found differ by a few mutational steps and several of them were shared between different geographical areas. On the other hand, several conserved regions within PvSIAP2 were pre- dicted as potential B-cell or T-cell epitopes. Considering these characteristics and its role in hepatocyte invasion, the PvSIAP2 protein emerges as a promising antigen to be considered in a multi-antigen-multi-stage (multiva- lent) fully effective vaccine against *P. vivax* malaria.

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INVESTIGATING APPROACHES TO INCREASE UPTAKE OF MALARIA VACCINE AND OTHER CHILDHOOD HEALTH INTERVENTIONS IN WESTERN KENYA: A FEASIBILITY STUDY

Nelli Westercamp[1], Prisca Adhiambo Oria[2], Benard Omondi Ochieng[2], Daniel McDermott[3], Oliver Omondi Towett[2], Victoria Seffren[1], Wycliffe Odongo[1], Titus Kimutai Kwambai[4], Sheila Muya Ekodir[2], Brian Louis O. Seda[2], Julie R. Gutman[1], Sarah G. Staedke[3], Lauren Cohee[3], Jacqueline Osido[5], Rose Jalang'o[6], Simon Kariuki[2]

[1]CDC, Atlanta, GA, United States, [2]Kenya Medical Research Institute, Kisumu, Kenya, [3]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [4]CDC, Kisumu, Kenya, [5]Rarieda Subcounty Ministry of Health, Siaya, Kenya, [6]Ministry of Health, National Vaccines & Immunisation Program, Nairobi, Kenya

Kenya was among the first countries to introduce the RTS,S/AS01 (RTS,S) malaria vaccine. While first-dose coverage exceeds 80%, completion of the 4-dose schedule, particularly dose 4 (RTS,S-4) at 24 months, remains low (~33%). Similar drop-offs occur for other second-year-of-life interventions. As part of the Malaria Vaccine Learning Agenda, this study evaluated strategies to improve RTS,S-4 uptake and integration with other child health services. A mixed-methods implementation science study was conducted in 8 health facilities in Rarieda sub-county, Siaya County, western Kenya (August-December 2025). Four interventions were developed using human-centered design: 1) insecticide-treated net provision at RTS,S-4 visits; 2) community health promoter (CHP)-led household immunization tracking and referral; 3) SMS-enabled defaulter tracing; and 4) immunization systems strengthening (facility workflows and tools). Evaluation used facility data, program logs, and qualitative interviews. CHPs screened >2,200 children in a month; 35-39% were under-vaccinated and referred, with ~60% completing referrals within two weeks. A total of 585 children received any RTS,S, with 64-80% of doses delivered past schedule, indicating backlog catch up; >50% were overdue for RTS,S-4. RTS,S-4 delivery increased 4-15× versus historical averages and 2-7× versus facility targets. Co-delivery was high, with 68-100% of RTS,S-4 provided alongside other services (MR2, vitamin A, deworming). Intervention adoption was strong, with CHPs central to performance. Implementation was feasible using simple tools, though system shocks (e.g., health worker strike) disrupted continuity. Multicomponent interventions showed strong early signals of feasibility, acceptability, and effectiveness in improving RTS,S uptake, largely through catch-up of overdue children. CHP-led tracing, combined with simple facility systems and caregiver reminders, was critical. Integration with broader child health services improved efficiency and caregiver value. System reliability (staffing, supplies, service continuity) remains key for sustainability.

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ACCELERATING MALARIA VACCINE DOSE COMPLETION IN HARD-TO-REACH COMMUNITIES THROUGH SUSTAINED CAREGIVER FOLLOW UP AND VACCINE SERVICE LINKAGE BY HOUSE-TO-HOUSE MOBILIZERS IN BAYELSA, NIGERIA

Ekpo Edet[1], Ayebatari Lawson[1], Hadiza Jibril[1], Solomon Onah[1], Yvette Collymore[2], Tara Petronio[2], John Tanko Bawa[3], Garba Rufai[4], Nnenna Ogbulafor[5], Appah Weri[6], Gbanai Onikari[6]

[1]PATH, Abuja, Nigeria, [2]PATH, Seattle, WA, United States, [3]PATH, Accra, Ghana, [4]National Primary Health Care Development Agency, Abuja, Nigeria, [5]National Malaria Elimination Programme, Abuja, Nigeria, [6]Bayelsa State Primary Health Care Board, Bayelsa, Nigeria

Nigeria contributes significantly to global malaria cases and deaths. To help reduce the disease's burden, a malaria vaccine is being phased in to complement existing prevention interventions. In Bayelsa and Kebbi States, where the vaccine was first introduced in December 2024, completion of the four-dose vaccine schedule remains a challenge. One approach to increasing uptake of all doses and reducing dropout rates has been the reactivation of sustained household visits by house-to-house mobilizers who identify children who missed doses for referral to health facilities for vaccination. PATH, in consultation with the National Primary Health Care Development Agency, is providing technical assistance in two low-performing Local Government Areas (LGAs) in Bayelsa State (Ogbia and Sagbama) to equip mobilizers with interpersonal communication skills and on-the-job mentoring for house-to-house visits in hard-to-reach, mostly riverine communities. The mobilizers inspect child health cards, remind caregivers of upcoming vaccination appointments, reinforce full-dose completion, and identify children who missed doses for referral. Between October 2025 and February 2026, mobilizers conducted more than 1,260 visits to households. As a result, an additional 18,526 children in Bayelsa State received malaria vaccine, with dropout rates declining from 21 percent to 16 percent between the first and second doses and from 32 percent to 25 percent between the first and third doses. Sustained house-to-house mobilization and community-based delivery significantly improved malaria vaccine uptake and reduced dropout in the state. By embedding continuous community-based follow-up within routine immunization systems, immunization programs can accelerate full-dose completion and maximize vaccine impact.

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RATIONALE AND PRECLINICAL STUDIES SUPPORTING CLINICAL DEVELOPMENT OF WRAIR'S NEXT-GENERATION MALARIA VACCINE CANDIDATE - FMP015/ALFQ

Sheetij Dutta[1], Dallas Brown[1], Shreeram Nallar[1], Aziza Frank[1], Shelby Foor[1], Jessica Bolton[2], Yevel Flores-Garcia[3], Fidel P. Zavala[4], William Fugina[1], Elke S. Bergmann-Leitner[5], Alison Roth[6], Nelson Michael[1], Evelina Angov[6], Mangala Rao[7], Gary Matyas[1]

[1]WRAIR, Silver Spring, MD, United States, [2]Henry M Jackson Foundation/ NMRC, Silver Spring, MD, United States, [3]Johns Hopkins Univ, Baltimore, MD, United States, [4]Johns Hopkins Univ Sch of Pub Hlth, Baltimore, MD, United States, [5]Walter Reed Army Institute, Silver Spring, MD, United States, [6]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [7]Silver Spring, MD, United States

Globally 282 million cases and 610,000 deaths were attributed to malaria in 2024; and despite the availability of drugs to prevent infection, 30 malaria cases were diagnosed among the United States service members who travelled to malaria endemic areas in 2024. While the RTS,S and R21 vaccinations are now being implemented to reduce childhood mortality, no vaccine is approved to prevent disease among semi-immune adults who serve as the parasite reservoir, highly susceptible pregnant women and immunologically naive traveler populations. Next-generation vaccines are being developed to not only reduce cost of goods but to improve upon the efficacy and durability of the currently available malaria vaccines. WRAIR's Next-Gen FMP015 vaccine was developed to overcome rapid waning immunity and to recapitulate the high affinity and inhibitory activity of NPNA repeat specific monoclonal antibodies against Circumsporozoite Protein (CSP). Tobacco Mosaic virus-like particle (VLP) - based immunogens were developed using reverse-vaccinology and were compared to the benchmark vaccines RTS,S and FMP013 (WRAIR's first generation CSP vaccine). WRAIR's FMP015 immunogen evaluated in mice induced high levels of CSP monoclonal antibody equivalents, measured by the CBASQE assay and was found approach ~3X qualitative improvement over the RTS,S and 10-fold improvement over WRAIR's FMP013 vaccine in the rodent challenge models. In rhesus, the FMP015 formulated with WRAIR's ALFQ adjuvant was found to be safe and immunologically superior to the benchmark vaccines. This non-human primate study also allowed us to down-select the dose of FMP015/ALFQ that can achieve a balanced high titer, avidity, durability and high monoclonal antibody equivalents. A scalable process for the manufacture of a clinical batch of FMP015 antigen has been developed and scale-up manufacture is underway. The TMV-based FMP015 vaccine received a favorable pre-IND review from the US FDA. In addition to the preclinical rationale, a clinical development plan for advancing FMP015/ALFQ to the field will be discussed.

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ANALYSIS OF THE RTS,S MALARIA VACCINE IN AFRICAN CHILDREN IDENTIFIED TWO NOVEL *PLASMODIUM FALCIPARUM* TARGETS OF PROTECTIVE ANTIBODY RESPONSES.

Alessia Hysa[1], D. Herbert Opi[1], Joshua Waterhouse[1], Sandra Chishimba[1], Jessica L. Horton[2], Natalie Kingston[3], Hans J. Netter[4], David Wetzel[5], Michael Piontek[6], Gaoqian Feng[7], Jahit Sacarlal[8], Carlota Dobano[9], Liriye Kurtovic[1], James Beeson[1]

[1]Burnet Institute, Melbourne, Australia, [2]University of Oxford, Oxford, United Kingdom, [3]University of Leeds, Leeds, United Kingdom, [4]RMIT University, Melbourne, Australia, [5]Miltenyi Biotec, Bergisch Gladbach, Germany, [6]ARTES Biotechnology, Langenfeld, Germany, [7]Nanjing Medical University, Nanjing, China, [8]Manhica Health Research Center, Maputo, Mozambique, [9]Barcelona Institute for Global Health (ISGlobal), Barcelona, Spain

The RTS,S vaccine provides modest and short-lived efficacy against malaria in children (~55% over 12-months). Vaccine-induced immunological correlates of protection are poorly defined, hindering progress towards developing highly effective next-generation vaccines. RTS,S targets the *Plasmodium falciparum* circumsporozoite protein (CSP) which includes several epitopes composed of repeated NANP or NVDP amino acid sequences or junctional (KQPADGNPDPNANPN amino acids) sequences. The RTS,S construct itself includes only NANP amino acids and excludes the NVDP and junctional epitopes that were recently identified as targets of potent inhibitory monoclonal antibodies. NVDP and junctional epitopes share some sequence similarity with NANP amino acid sequences, and we therefore hypothesised that RTS,S-induced antibodies would also recognise these important epitopes. We investigated the fine specificity of RTS,S-induced antibodies to six peptides representing epitopes comprising NANP repeats, NVDP repeats and the junction amino acid sequence and evaluated RTS,S-induced antibody responses to each peptide to identify novel immunological correlates of protection. We measured responses in a large RTS,S phase 2 clinical trial in young children 1-4 years old (n=735). We found that a subset of children generated IgG with specificity for a short peptide composed of few NANP repeats and cross-reactivity to the junction peptide representing an epitope excluded from the vaccine construct. Notably, over a follow-up period of 18-months, children with high IgG responses to these peptides had a significantly reduced risk of clinical malaria, compared to children with low responses. These findings reveal promising new correlates of protection for the RTS,S vaccine which could be used to inform the development of highly effective next-generation CSP-based vaccine constructs and as biomarkers of protection to evaluate vaccines in future clinical trials.

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ABO BLOOD GROUP AND ANTIBODY RESPONSES TO RTS S/AS01E MALARIA VACCINE IN CHILDREN IN CAMEROON

Valentina Josiane Epse Essomba Ngo Bitoungui[1], Sorelle Tchuigue Ndakou[2], Leonelle Andrea Nyoumoue[3], Arthur Jordan Vessoulam Vessah[3], Abel Lissom[4], Hamsatou Hadja[5], René Ghislain Essomba[6]

[1]University of Dschang, Department of Microbiology, Hematology and Immunology, Faculty of Medicine an, Dschang, Cameroon, [2]School of Health Sciences of the Catholic University of Central Africa, Yaounde, Cameroon, [3]School of Health Sciences of the Catholic University of Central Africa, Yaoundé, Cameroon, [4]University of Bamenda/ National Public Health Laboratory, Yaounde, Cameroon, [5]1National Public Health Laboratory, Yaoundé, Cameroon, [6]Faculty of Medicine and Biomedical Sciences, University of Yaounde 1, Yaoundé, Cameroon

Malaria remains a major public health challenge in Cameroon, particularly among children under five years of age. The introduction of the RTSS/AS01E vaccine into the national immunization program represents an important step forward in malaria control; however, little is known about how individual factors, such as ABO blood group, may influence the immune response. This study aimed to assess whether anti-circumsporozoite protein (CSP) IgG and IgM antibody responses to RTSS/AS01E vary by ABO blood group in children in Cameroon. We conducted a prospective cohort study among 208 children aged 6 to 12 months in the Centre (n=74), Littoral (n=54), and West (n=84) regions. Participants were recruited at routine vaccination visits and received RTSS/AS01E at 6 (V1), 7 (V2), and 9 (V3) months of age. After parental consent, demographic and clinical data were collected, and blood samples were obtained at each visit for malaria diagnosis by microscopy and quantification of anti-CSP IgG and IgM antibodies using ELISA. Statistical analyses were performed using repeated measures approaches in GraphPad Prism and Microsoft Excel. The mean age was 6.4 ± 2.2 months, with a male to female ratio of 1.2. Blood group distribution was O (n=99; 47.7%), A (n=53; 25.6%), B (n=44; 21.1%), and AB (n=12; 5.6%), with 97.4% being Rhesus positive. Seroprevalence of anti-CSP antibodies was 94.2% for IgM and 96.3% for IgG, and antibody levels increased significantly across vaccine doses ($p<0.0001$). No statistically significant differences in IgG or IgM responses were observed between ABO blood groups, though descriptive trends suggested higher increases in O, A, and B compared to AB. Malaria infection prevalence during follow-up did not differ significantly by blood group, although cases decreased in group O and slightly increased in A, B, and AB. These findings indicate that RTSS/AS01E induces strong humoral immune responses in

Cameroonian children, independent of ABO blood group, and highlight the need for further studies with larger cohorts and controlled confounding to better understand host factors influencing malaria vaccine immunogenicity.

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MALARIA VACCINE UPTAKE AND COMPLETION IN OLDER CHILDREN: IMPLICATIONS FOR EMERGENCY VACCINE DELIVERY IN WESTERN KENYA, 2026

Anh Ngoc Ly[1], **Youngjoo Park**[1], Cyrus Kagecha[2], Damaris Lagat[2], Milkah Akala[2], Dominic Ouma[2], William Mulwa[2], Felister Mueni[2], Joshua Ondiege[3], Christina Mwachari[2], Sam Wafula[2], Rose Jalang'o[4], Pierre Muhoza[1], Emily Koech[2], Ciara Sugerman[1]

[1]U.S. Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Centre for International Health, Education and Biosecurity, Nairobi, Kenya, [3]Siaya County Department of Health and Sanitation, Nairobi, Kenya, [4]National Vaccines and Immunization Program, Ministry of Health, Nairobi, Kenya

Vaccination platforms reaching children beyond infancy are critical for delivery of multi-dose vaccines and for preparedness to deploy vaccines during public health emergencies. In Kenya, the malaria vaccine (RTS,S) was introduced in 2019 as a four-dose series at 6, 7, 9, and 24 months of age through routine immunization (RI). During 2023-2024, catch-up vaccination (vaccinating eligible children missing recommended doses) for all antigens through RI was strengthened in Siaya County, Western Kenya via facility-level process improvements, such as strengthening immunization screening and referral, defaulter tracing, and record documentation. We assessed RTS,S uptake and series completion in Siaya and Migori (comparator county in Western Kenya). From January - March 2026, we conducted health facility exit interviews at 32 facilities in both counties among mothers of children aged 24-59 months using convenience sampling. We abstracted children's vaccination histories from home-based records (HBR) during health facility exit interviews when available. We calculated the proportion of children vaccinated with all doses of RTS,S and dropout rates from dose 1 (RTS,S1) to dose 4 (RTS,S4) to describe the proportion of children who started but did not complete the RTS,S series. In Siaya, 130 HBRs were reviewed. The proportion of children vaccinated with RTS,S1, RTS,S3, and RTS,S4 were 71.5%, 70.0%, and 69.2%, respectively; 3.2% of children who received RTS,S1 did not receive RTS,S4. In Migori, 148 HBRs were reviewed. The proportions of children vaccinated were 60.1% for RTS,S1, 55.4% for RTS,S3, and 38.8% for RTS,S4; 37.1% of children received RTS,S1 and not RTS,S4. Higher coverage and substantially lower dropout in Siaya compared with Migori suggest benefits of strengthened catch-up vaccination strategies. However, high dropout in Migori highlights persistent gaps in reaching children in the second year of life, with implications for delivery of multi-dose vaccines and vaccines used during public health emergencies.

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TECHNICAL ASSISTANCE FOR QUALITY MALARIA VACCINE ROLLOUT IN THE DEMOCRATIC REPUBLIC OF CONGO (DRC): TRANSITIONING FROM A SINGLE PROVINCE TO A NATIONAL SCALE

Djeneba Coulibaly Epse Traore[1], Leon Kinuani[2], Audry wa Kamba[2], Baudouin Baangi Matela[3], Godefroid mpanya[1], Crispin Kazadi[2], Anne Boher[4], John Bawa[5], Yvette Collymore[6], Saira Nawaz[6]

[1]PATH, Kinshasa Blvd du 30 Juin, Republic of the Congo, [2]MoH, Kinshasa, Republic of the Congo, [3]General Secretary of Public Health, DRC, Durham, NC, United States, [4]Bruxelles, Belgium, [5]PATH, Accra, Ghana, [6]PATH, Washington, DC, United States

In the Democratic Republic of Congo (DRC), malaria remains the leading cause of morbidity and mortality among children under five. In 2024, the country introduced the malaria vaccine as a complement to existing prevention and treatment strategies, with the aim of vaccinating 4.8 million children aged 6 to 23 months in high- and medium-burden areas. The rollout adopted a phased approach, starting in October 2024 in Kongo Central Province. Drawing on a KAP survey conducted that year in nine provinces, PATH assisted the Expanded Program on Immunization (EPI) and the Ministry of Health, Hygiene, and Prevention to implement a deep-dive model across 31 health zones. The technical assistance aimed to support malaria vaccine uptake, optimize coverage of all four malaria vaccine doses, strengthen factors positively influencing uptake across all antigens, and improve immunization service quality, without compromising other malaria interventions. Support was structured across five pillars: coordination and planning, service delivery quality, data use, vaccine stock management and community engagement, including health worker communication and outreach, with an intentional focus on engagement of women as primary caregivers and men as decision-makers, to strengthen schedule adherence. Lessons were systematically translated to inform ongoing vaccine deployment across health zones and provinces. When the malaria vaccine was introduced in 21 additional provinces in 2025 and 2026, PATH drew on learnings from Kongo Central Province to assist EPI in deploying a lighter-touch approach to expand reach, strengthen coordination, and enable more efficient use of limited resources, while retaining a strong focus on learning and quality. This abstract presents how this model, developed from rigorous field evidence in the lead province, was adapted and scaled to support quality malaria vaccine rollout across 22 provinces, demonstrating the value of a learning-driven, systems-embedded approach to vaccine introduction in one of the world's malaria highest-burden countries.

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WEEKLY DATA QUALITY AUDITS FOR THE MALARIA VACCINE IN THE DEMOCRATIC REPUBLIC OF CONGO (DRC): CHALLENGES, RESULTS, AND LESSONS LEARNED

Constant Kingongo[1], Djeneba Coulibaly-Traoré[1], Saira Nawaz[2], Guylain Sundisa[1], Liliane Pambu[3], Joel Mbula[3], Anne Boher[4], Mymy Mwika[5]

[1]PATH, Kinshasa, Democratic Republic of the Congo, [2]PATH, Washington DC, WA, United States, [3]Ministry of Health, Kongo Central, Democratic Republic of the Congo, [4]PATH, Bruxelles, Belgium, [5]Ministry of Health, Kinshasa, Democratic Republic of the Congo

In the Democratic Republic of Congo, the introduction of the malaria vaccine in October 2024 required a reliable information system to monitor vaccination coverage. Early analyses revealed significant discrepancies between vaccination registries, checklists, reports from the National Health Information System, and DHIS2 data. These discrepancies compromised the quality of the reported data. In response, weekly data quality audits (DQAs) were implemented in seven districts in Kongo Central: Muanda, Boma, Boma Bungu, Kitona, Kisantu, Mbanza-Ngungu, and Nginga. These assessments support healthcare providers and verify the consistency between data sources, the completeness of key indicators (MV1-MV3, DTC1, DTC3, and MCV1), and the quality of DHIS2 entries. The Verification Factor (VF), which measures the discrepancy between reported and source data with an acceptable range of [95%-105%] according to WHO standards, serves as the primary performance indicator. For each discrepancy detected, the center's team immediately develops a corrective action plan, which may include individual coaching, update tools, and strengthen report transmission channels. The DQAs enabled the rapid detection of irregularities and a gradual decrease in the VF, reflecting a measurable improvement in data accuracy. The consistency of the MV1-MV3 indicators and the completeness of the DHIS2 reports have improved, with the fastest progress occurring in health centers that receive close support. However, persistent challenges remain, including inadequate data collection tools, limited connectivity in remote districts, and high provider workloads. This approach has proven effective in improving the reliability of routine immunization and malaria vaccination data in a resource-limited setting. The lessons learned offer a model that can be adapted to other vaccine rollouts requiring rigorous, continuous monitoring of data quality.

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ENGAGING NON-TRADITIONAL PARTNERS TO STRENGTHEN MALARIA VACCINE UPTAKE ACROSS SIX PROVINCES IN THE DEMOCRATIC REPUBLIC OF CONGO (DRC): A GOVERNMENT-LED CO-CREATION APPROACH

Anne Boher[1], Djeneba Coulibaly Traore[2], Christophe Konso[3], Jennyfer Tamba Nkebani[2], Godefroid Mpanya[2], Karine Mianda[3], Yvette Collimore[4]

[1]PATH, Bruxelles, Belgium, [2]PATH, Kinshasa, Democratic Republic of the Congo, [3]MoH, Kinshasa, Democratic Republic of the Congo, [4]PATH, Washington D.C., DC, United States

The DRC introduced the malaria vaccine in October 2024, expanding to 22 provinces by March 2026. Despite the encouraging initial uptake, with 15 of 16 provinces surpassing the 70% national target by 2025, the primary challenge lies in sustaining the program's momentum across the four-dose schedule. A KAP survey conducted in early 2024 found that only 50% of caregivers understood the correct dose schedule, and 31% feared community or family disapproval. To address these challenges, PATH provided technical support to the Ministry of Health, Hygiene and Prevention to design a Non-Traditional Partner co-creation approach. The strategy was initially implemented in the lead province of Kongo Central and was expanded to 22 districts across 6 provinces. A five-step methodology guided implementation: Community influencers were mapped; Participatory analysis of obstacles to uptake was conducted; Challenges were prioritized based on local relevance; Context-specific strategies were designed; commitments were formalized through signature. A series of workshops were held, bringing together representatives from schools, faith groups, local media, women's associations and youth clubs. Participants developed community mobilization strategies, addressing the complexity of the four-dose vaccination schedule. The proposed actions included parent engagement, radio broadcasts, community dialogues, and integration of messages into religious and cultural events. Coordination frameworks monitored and aligned implementation with provincial health objectives. By March 2026, 320 groups and associations had been enrolled on a voluntary basis. They collaborate closely with community-based health workers and youth clubs, establishing an integrated, multi-level outreach network. This government-led, evidence-informed model offers a scalable framework for strengthening demand for primary health care, inc. malaria prevention tools such as Long-Lasting Impregnated Mosquito Nets in complex, resource-limited settings.

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IMMUNOGENICITY AND PROTECTIVE EFFICACY OF A SYNTHETIC BLOOD-STAGE *PLASMODIUM VIVAX* MALARIA VACCINE

Francis B. Ntumngia[1], Gregory Howard[2], Samantha Jones Barnes[3], Shriya Yavasani[1], Pooya Mahdavi[1], Hai-Quan Mao[4], John Adams[1]

[1]Univ of South Florida, Tampa, FL, United States, [2]John Hopkins University, Baltimaore, MD, United States, [3]Univ of South Florida, Lutz, FL, United States, [4]Johns Hopkins University, Baltimore, MD, United States

The *Plasmodium vivax* Duffy binding protein (DBP) is a leading blood-stage vaccine candidate, since it plays an essential role in the infection of reticulocytes. Naturally acquired anti-DBPII antibodies can block *P. vivax* merozoite invasion of reticulocytes and are associated with protection against disease. Despite its functional role in the invasion process, allelic variation in dominant B-cell epitopes of DBPII are associated with strain-specific immunity, which is a major challenge to vaccine efficacy. An engineered rDBPII based protein, DEKnull-2, with altered variant B-cell epitopes, retains conserved, neutralizing epitopes and is reactive with long-term memory B-cells and stable binding inhibitory antibodies (BIAbs) from natural *P. vivax* infections. This study evaluated the immunogenicity of a rDEKnull-2 nanoparticle vaccine (NPV) in BALB/c mice. The vaccine was highly immunogenic mice, eliciting cross-reactive antibodies against naturally occurring variant rDBPII alleles. Most importantly, the DEKnull-2 NPV elicited significantly higher inhibitory antibodies against merozoite invasion of human erythrocytes in in vitro blood-stage parasite cultures than the reference DBPII-Sal1 vaccine strain. This data provides support for DEKnull-2 as a blood-stage *P. vivax* vaccine and validates the suitability of NPVs as a delivery system for a DBP based vaccine compared to traditional subunit vaccine formulations.

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GENOMIC EPIDEMIOLOGY AND MACHINE LEARNING CLASSIFICATION OF FLUOROQUINOLONE RESISTANCE-ASSOCIATED SUBLINEAGES IN *SALMONELLA TYPHI* FROM NIGERIA

Joy Amarachi Okebugwu

National Institute of Pharmaceutical Research and Development, Federal Capital Territory, Nigeria

Typhoid fever caused by multidrug-resistant (MDR) *Salmonella Typhi* is an escalating public health burden in Nigeria, where ciprofloxacin remains first-line treatment despite increasing failure. However, Nigeria-specific genomic surveillance data remain limited, constraining evidence-based policy. We analysed 104 *S. Typhi* genomes from Nigerian isolates (ENA: PRJEB6352). Reads were quality-filtered and mapped to the CT18 reference genome, AMR genes were identified using CARD, and genotyping was performed using GenoTyphi. QRDR mutations were extracted from variant calls. A Random Forest classifier was trained on 2,265 SNPs, excluding the *parC* locus to prevent circularity, using 5-fold cross-validation to classify isolates by *parC* S80R mutation status as a surrogate for high-level fluoroquinolone resistance. All isolates belonged to genotype 3.2.1 (confidence = 1.0), consistent with clonal expansion of a single introduced lineage that diversified locally into two genomically distinct resistance subpopulations. All isolates shared a uniform MDR gene profile comprising *bla*TEM-1, *catA1*, *dfrA14*, *sul2*, *aph(3")-Ib*, *aph(6)-Id*, *tet(B)* and *aac(6')-Iy, consistent with the ACSSuT pattern. All isolates carried *gyrA* S83Y, while 63.5% (66/104) also harboured *parC* S80R associated with high-level resistance. PCA showed clear genomic separation of *parC* S80R carriers along PC1 (51.5% variance), reflecting within-lineage divergence. The model achieved AUROC 1.00, accuracy 99.0% and F1-score 99.3%, identifying sublineage-specific genomic signatures for targeted surveillance. These findings indicate widespread fluoroquinolone resistance and risk of ciprofloxacin failure, supporting updated treatment guidelines and expanded typhoid conjugate vaccine deployment.

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HOUSEFLIES (*MUSCA DOMESTICA*) AS RESERVOIRS OF ANTIBIOTIC-RESISTANT BACTERIA: A COMPARATIVE MICROBIAL ANALYSIS IN PERI-URBAN ENVIRONMENTS

Ama Nyameyie Pinkrah, Ohemaa Osei, Prince Osei, Rhoda Coffie, Dorcas Owusu

Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

Houseflies (*Musca domestica*) are mechanical vectors that transmit a wide range of microbial pathogens, including antibiotic-resistant bacteria. Their close association with human and waste environments makes them important contributors to the spread of infectious diseases. This study investigated the microbial profile and antimicrobial resistance patterns of bacteria associated with houseflies collected from different ecological settings within the Kwame Nkrumah University of Science and Technology, Ghana. A total of 300 houseflies were collected from three distinct environments: market, farm, and student residential halls. External and internal microbial loads were assessed by washing flies in sterile saline, then homogenizing whole specimens. Bacterial isolates were cultured using standard microbiological techniques and identified through Gram staining, microscopy and biochemical tests. Antimicrobial susceptibility testing was done using standard protocols to determine resistance patterns. Houseflies from the market showed the highest bacterial load (353.75 CFU/mL), then those from the farm (323.25 CFU/mL) and residential halls (275.75 CFU/mL), indicating increased microbial contamination in high

human-activity areas. Notably, *Micrococcus spp*. isolated from residential halls demonstrated multidrug resistance to all 12 antibiotics tested, while *Staphylococcus spp.* also showed considerable resistance. In contrast, isolates from the farm environment such as *Enterococcus* spp. and *Micrococcus* spp. demonstrated a comparatively high level of antibiotic susceptibility. The findings highlight the role of houseflies as potential reservoirs and vectors of antibiotic-resistant bacteria, particularly in densely populated and poorly sanitized environments. The high bacterial load and resistance patterns observed in market-associated flies underscore the need for improved environmental hygiene, waste management, and continuous microbial surveillance. This study reinforces the public health importance of controlling fly populations to reduce the dissemination of antibiotic-resistant pathogens.

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EVALUATION OF THE PYRAZINAMIDE RESISTANCE PHENOTYPE IN H37RV *MYCOBACTERIUM TUBERCULOSIS* STRAINS WITH DOUBLE DELETION OF CANDIDATE METALLOCHAPERONE GENES IMPLICATED IN PYRAZINAMIDE ACTION

Antony Barrientos Zevallos[1], Fresie Denisse Maciel Carpio[2], Mishell Landa Flores[3], Clarisse Cornejo[1], Patricia Sheen[4], Mirko Zimic[1]

[1]Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Lima, Peru, [3]Mishell, SANTA ANITA, Peru, [4]Cayetano Heredia University, LIMA, Peru

Pyrazinamide resistance remains a major challenge for tuberculosis treatment, particularly in multidrug-resistant tuberculosis, and understanding the mechanisms that modulate pyrazinamide activity is essential for improving its clinical utility. Although pyrazinamide activation depends on pyrazinamidase, the contribution of accessory proteins, including candidate metallochaperones such as Rv2059, Rv0944, Rv2102, and Rv1852, remains unclear. In this study, we aimed to generate double knockouts in Mycobacterium tuberculosis H37Rv using the ORBIT strategy to investigate whether simultaneous deletion of these genes could provide a basis for evaluating their role in pyrazinamide action. A sequential ORBIT approach was optimized by testing electroporation, selection, and post-electroporation recovery conditions, enabling recovery of candidate colonies for the MKO_Rv2059_Rv2102, MKO_Rv2059_Rv0944, and MKO_Rv2059_Rv1852 events. Preliminary molecular validation by PCR of lateral junctions showed amplification patterns compatible with both recombination ends in 7 of 8 clones for MKO_Rv2059_Rv2102; in most clones for MKO_Rv2059_Rv0944, with one discordant clone; and left-junction positivity in 6 of 11 clones for MKO_Rv2059_Rv1852. Amplicon sequencing using Oxford Nanopore further confirmed selected clones consistent with the expected constructs for MKO_Rv2059_Rv2102 and MKO_Rv2059_Rv0944. These preliminary findings indicate that sequential ORBIT is a feasible strategy for generating double knockouts in M. tuberculosis and provide an experimental platform for future studies evaluating the phenotypic effect of candidate metallochaperones on pyrazinamide susceptibility.

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ASSESSMENT OF FACTORS INFLUENCING PERCEPTIONS OF ANTIBIOTIC PRESCRIBING AND ANTIBIOTIC RESISTANCE AMONG PERUVIAN PHYSICIANS

Jose Camones-Huerta[1], Coralith Garcia[2], Fiorella Krapp[2], Claudia Rondon[1], Esther Kunzli[3]

[1]School of Medicine, Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Instituto de Medicina Tropical Alexander von Humboldt, Lima, Peru, [3]Swiss Tropical and Public Health Institute, Allschwil, Switzerland

Antimicrobial resistance (AMR) is an increasing global public health threat. Strategies such as antimicrobial stewardship programs (ASP) aim to mitigate this issue; however, understanding physicians' perceptions of antibiotic use is essential to strengthen these interventions. This is the first national study assessing these perceptions in Peruvian hospitals, providing strategic evidence to inform ASP-related policies. This study evaluated physicians' perceptions of antibiotic use, AMR, and factors influencing antibiotic prescribing across 10 hospitals in Peru. Physicians were surveyed using a 9-point Likert scale assessing agreement with statements on antibiotic use and prescribing determinants. Latent class analysis (LCA) and multinomial regression were performed to identify prescribing patterns. A total of 431 physicians participated; 88.34% (n=379) and 85.50% (n=366) strongly agreed that AMR is a global and national problem, respectively. Three latent classes were identified for self-perception of antibiotic use: conscious but uncertain prescribers, high antibiotic prescribers, and balanced and prudent prescribers. Regarding factors influencing prescribing, three latent classes were identified: physicians influenced by regular training, clinical guidelines, and laboratory results. Significant differences ($p<0.05$) were observed between latent classes and professional characteristics, including hospital, role (attending vs resident), and years of experience. Physicians' perceptions of antibiotic prescribing vary significantly according to professional role, institutional setting, and experience. These findings provide a critical foundation for designing targeted, system-level interventions to strengthen rational antibiotic use and optimize antimicrobial stewardship strategies in Peru.

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ACID-DEPENDENT TRANSCRIPTIONAL INTERACTIONS AMONG CANDIDATE PYRAZINAMIDASE METALLOCHAPERONES IN *MYCOBACTERIUM TUBERCULOSIS*

Carlos Alonso Flores Bancayan[1], Fresie Denisse Maciel Carpio[1], Mishell Landa Flores[1], Gianella Disareine Pittman Castro[1], Gina Lobaton Almonacid[1], Mirko Zimic[1], Patricia Sheen[2]

[1]Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Universidad Peruana Cayetano Heredia, LIMA, Peru

Tuberculosis remains a major global health problem, and pyrazinamide (PZA) requires conversion to pyrazinoic acid (POA) by pyrazinamidase. Previous studies from our group showed that single knockout of *Rv2059*, *Rv2102*, or *Rv0944* reduced POA efflux and increased PZA resistance up to two-fold vs wild-type; *Rv2059* showed the strongest efflux defect. Exploratory analysis of *Rv1852* also showed partial POA efflux reduction and decreased PZA susceptibility at pH 6.8. We therefore hypothesized an acid-dependent transcriptional network centered on *Rv2059*. To determine whether deletion of one candidate altered expression of the others, reciprocal gene expression was evaluated by RT-qPCR in *Mycobacterium tuberculosis* H37Rv wild-type and single-knockout strains at pH 6.8 and 6.2, with and without PZA, using *sigA* as reference and Pfaffl-based relative quantification. At pH 6.8, knockout of *Rv2059* increased *Rv1852* without and with PZA (0.199 vs 0.052, p=0.0066; 0.212 vs 0.076, p=0.0162) and increased *Rv0944* only without PZA (0.126 vs 0.066, p=0.0034); only knockout of *Rv1852* increased *Rv2059*, without and with PZA (0.454 vs 0.108, p=0.0009; 0.579 vs 0.141, p=0.0023). At pH 6.2, knockout of *Rv2059* decreased *Rv0944* without and with PZA (0.215 vs 0.457, p=0.0465; 0.199 vs 0.478, p=0.0052) and reduced basal *Rv1852* (0.134 vs 1.24, p=0.0012); PZA increased *Rv1852* only in Δ*Rv2059* (0.635 vs 0.134, p=0.0344). Knockout of *Rv2102* decreased *Rv2059* only without PZA (0.529 vs 1.48, p=0.0006), whereas knockout of *Rv0944* and *Rv1852* decreased *Rv2059* without and with PZA (0.212 vs 1.48, p=0.0006; 0.286 vs 1.38, p=0.0064; 0.400 vs 1.48, p=0.0003; 0.517 vs 1.38, p=0.0039). These findings support acid-dependent transcriptional interactions among candidate pyrazinamidase metallochaperones and show that *Rv2059* exhibits the strongest and most consistent transcriptional associations across single-gene knockout strains, whereas *Rv0944* and *Rv1852* show broader effects than *Rv2102* under the conditions tested.

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IMPLEMENTATION AND IMPACT OF ANTIMICROBIAL STEWARDSHIP PROGRAMS IN PAKISTAN: A SCOPING REVIEW (2020 TO 2026)

Ghania Nadeem

Sameera medical center, Jeddah, Saudi Arabia

Antimicrobial resistance (AMR) is a major global health threat, disproportionately affecting low- and middle-income countries (LMICs) such as Pakistan. Antimicrobial stewardship programs (ASPs) are critical interventions to optimize antibiotic use and mitigate resistance; however, their implementation in resource-constrained settings remains variable. This study aimed to systematically map recent evidence on ASP implementation and effectiveness in Pakistan. A scoping review was conducted using established methodological frameworks. Electronic databases were systematically searched for studies evaluating ASPs in Pakistan from 2020 to 2026. Eligible studies included hospital- and community-based interventions assessing antibiotic prescribing practices, resistance patterns, and stewardship strategies. Data were extracted and synthesized narratively. A total of 21 studies were included. Most studies were conducted in tertiary care hospitals and evaluated interventions such as clinical guideline implementation, audit and feedback, and antibiotic restriction policies. ASP implementation was consistently associated with improved antibiotic prescribing practices and reductions in inappropriate antibiotic use. However, implementation remained constrained by key barriers, including limited resources, lack of trained personnel, absence of standardized national guidelines, and weak surveillance systems. Evidence from primary care and community settings was notably limited despite recent expansion in research. Overall, ASPs in Pakistan demonstrate effectiveness in improving antibiotic use but remain fragmented and predominantly hospital-based. Strengthening national stewardship policies, expanding implementation to primary care, and enhancing surveillance systems are essential to address AMR in Pakistan and similar LMIC settings.

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DETECTION AND PROFILING OF ANTIBIOTIC RESISTANCE AMONG BACTERIA ISOLATES FROM MAJOR BANK NOTES IN ASHANTI MAMPONG MARKET

Albert Akuamoah Amoako, Bismark Sarkodie Acheampong, Kofi Addo Agyapong

University of Skills Training and Entrepreneurial Development, Ashanti, Ghana

Antibiotic resistance (ABR) is a significant global public health challenge and frequently handled items such as currency notes can act as vehicles for the transmission of resistant bacteria. This study investigated bacterial contamination and antibiotic resistance patterns of isolates from Ghanaian banknotes circulating in Asante Mampong Market, a major commercial center in the Ashanti Region. A descriptive cross-sectional design was used, involving the aseptic collection of 50 banknotes of different denominations (GH₵1, GH₵5, GH₵10, GH₵20, and GH₵50) from vendors. Samples were processed using phosphate-buffered saline, cultured on selective media (MacConkey Agar and Salmonella-Shigella Agar), and incubated. Bacterial identification was performed using Gram staining and biochemical tests, while antibiotic susceptibility was determined using the agar well diffusion method against ten antibiotics. Results showed that all isolates *Escherichia coli* (31.1%), *Shigella spp.* (23.4%), *Klebsiella spp.* (22.4%), *Salmonella spp.* and *Enterobacter spp.* were Gram-negative bacilli, indicating fecal contamination. The GH₵5 denomination had the highest contamination (39.5%), while GH₵1 showed no growth. All isolates were fully sensitive to several antibiotics but showed high resistance to Chloramphenicol and Penicillin (60%) and moderate resistance to Amoxicillin (40%) and Gentamicin (20%). The study concludes that banknotes in Mampong Market are reservoirs of pathogenic and antibiotic-resistant bacteria, posing a public health risk and recommends improved hygiene, sanitation, cashless transactions, and further molecular studies.

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BRIDGING THE COMMUNITY SURVEILLANCE GAP FOR ANTIMICROBIAL RESISTANCE, BASELINE FINDINGS FROM A DIGITAL ANTIBIOTIC REPORTING INITIATIVE IN NIGERIA

Yemisi Bolanle Ogundare[1], Perekebina Gbabo[1], Peter Abah[1], Zimuzor Nwokolo[1], Lovette Metiboba[2], David Akpan[2], Silvia Kelbert[3], Tigistu Adamu[3], Adetiloye Oniyire[1], Nochiketa Mohanty[4]

[1]Jhpiego, Abuja, Nigeria, [2]eHealth Africa, Abuja, Nigeria, [3]Jhpiego, Baltimore, MD, United States, [4]Jhpiego, New Delhi, India

Antimicrobial resistance (AMR) in Nigeria is driven by inappropriate antibiotic use and circulation of substandard and falsified (SF) medicines, yet surveillance remains largely reactive and facility-based, with limited visibility into community markets. The Trinity Challenge-funded Community Access to Effective Antibiotics (Com-WATCH) project, implemented by Jhpiego, leverages digital tools for community-level surveillance of SF medicines and stock control across six Nigerian states.A mixed-method baseline assessment was conducted in south and north of Nigeria. Quantitative surveys assessed antibiotic-related knowledge, attitudes, provider practices, digital readiness, and willingness to use a reporting platform among community pharmacists, patent and proprietary medicine vendors, and agrovets. Qualitative key informant interviews and institutional surveys examined regulatory frameworks, pharmacovigilance systems, AMR surveillance, and pathways for detecting and responding to suspected SF antibiotics.Findings showed multiple antibiotic-related data systems (DHIS2/HMIS, logistics, pharmacovigilance, AMR laboratory surveillance, and regulatory databases); however, these remain fragmented with limited visibility into community and informal markets. Knowledge of antibiotic use and AMR was moderate (61.4%), attitudes were highly positive (90.5%), and provider stewardship practices were variable (76.1%). Digital readiness was high (73.9%), and willingness to use a reporting platform was very high (88.4%).These findings highlight priorities including integrating digital reporting tools to address fragmented data systems, leveraging high provider readiness for early detection of SF antibiotics, and strengthening linkages between frontline providers and regulators to enable proactive antimicrobial stewardship and AMR response.

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FUNCTIONAL CHARACTERIZATION OF EFFLUX PUMP CANDIDATE GENES RV3008 AND RV1667C IN PYRAZINAMIDE RESISTANCE IN MYCOBACTERIUM TUBERCULOSIS USING ORBIT MEDIATED GENE KNOCKOUT

Renato I. Fernandez Cornejo, Patricia Sheen Cortavarría

Universidad Peruana Cayetano Heredia, San Martín de Porres, Peru

Pyrazinamide (PZA) resistance in *Mycobacterium tuberculosis* remains a major challenge to tuberculosis treatment. While mutations in *pncA* account for a substantial proportion of this phenotype, the presence of resistant strains lacking alterations in canonical genes suggests additional mechanisms, including pyrazinoic acid (POA) efflux. Previous studies identified Rv3756c, Rv3008, and Rv1667c as candidate efflux pump genes associated with PZA/POA resistance, whereas partial repression using CRISPRi failed to produce detectable phenotypic changes, suggesting that incomplete inhibition may underestimate their functional contribution. Here, we evaluated the role of Rv3008 and Rv1667c through individual gene deletions using ORBIT in the H37Rv strain. Knockouts were confirmed by PCR and sequencing. Fitness analysis showed that the growth rates of ΔRv3008 ($\mu = 0.01055\ h^{-1}$) and ΔRv1667c ($\mu = 0.01041\ h^{-1}$) did not differ significantly from the H37Rv control ($\mu = 0.01057\ h^{-1}$; $p > 0.05$). In contrast, both deletion strains exhibited a significant reduction in POA efflux rate compared to the control (ΔRv3008: 0.0149 vs 0.0175 $mM \cdot OD600^{-1} \cdot h^{-1}$, $p = 0.00414$; ΔRv1667c: 0.0144 vs 0.0175 $mM \cdot OD600^{-1} \cdot h^{-1}$, $p = 0.00019$),

with no significant difference between them. These results provide functional evidence that Rv3008 and Rv1667c contribute to POA efflux in *M. tuberculosis*. Susceptibility assays to PZA and POA under acidic pH conditions are currently underway to determine the impact of these deletions on the resistance phenotype. Overall, these findings support a possible alternative resistance mechanism mediated by efflux pumps and provide mechanistic evidence for PZA resistance.

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MODIFIABLE BURDEN OF BACTERIAL ANTIMICROBIAL RESISTANCE ASSOCIATED WITH HUMAN ANTIBIOTIC CONSUMPTION

Benjamin John Singer[1], Alec Gleason[2], Isabella Impalli[2], Mikkel D. Nagorsen[3], Chungman Chae[1], Lauren C. Granskog[4], Philip C. Lee[1], Ramanan Laxminarayan[5], Joseph A. Lewnard[1]

[1]University of California, Berkeley, Berkeley, CA, United States, [2]One Health Trust, Bengaluru, India, [3]University of California, San Francisco, San Francisco, CA, United States, [4]University of California, San Diego, San Diego, CA, United States, [5]Bangalore, KA, India

Human antibiotic use is widely believed to contribute to the evolution and maintenance of antimicrobial resistance (AMR) in bacterial pathogens—a recognition informing efforts to ensure antibiotics are used judiciously through stewardship interventions. However, these approaches encounter a critical tension in low-resource settings where gaps in antibiotic access persist. Quantitative understanding of the AMR burden likely to be avoidable through reducing the use of different antibiotic drugs is necessary to navigate competing demands to reduce AMR selection through antibiotic stewardship, and to mitigate bacterial disease mortality through expanded access. In analyses relating global surveillance data on AMR prevalence in 17 bacterial pathogens to estimated consumption across 7 antibiotic classes, the strongest evidence of use-resistance relationships arises in quinolones, aminoglycosides, penicillins, and other beta-lactam antibiotics. Across pathogens, evidence that AMR is associated with population-level antibiotic exposure is strongest for Acinetobacter spp., Salmonella spp., Streptococcus pneumoniae, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Klebsiella pneumoniae. We estimate that interventions achieving a 10% reduction relative to current levels of antibiotic use, across classes, could prevent 67,000 (95% confidence interval: 65,000-69,000) annual deaths attributable to AMR globally. Although substantial, this total represents only 0.87% of estimated bacterial disease mortality. In Sub-Saharan Africa, where risk of death due to bacterial disease is greatest, the avertible burden of AMR-attributable deaths comprises only 0.66% of bacterial disease mortality. Our findings suggest reductions in AMR-attributable mortality achievable through interventions mitigating antibiotic overuse may be modest relative to the persisting burden of antibiotic-preventable mortality. These estimates should inform assessments of the potential benefits and harms of restrictive versus permissive antibiotic use paradigms in epidemiologically-distinct settings.

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A CASE-CASE GENOME-WIDE ASSOCIATION STUDY (GWAS) IDENTIFIES HOST GENETIC SIGNALS IN PURINERGIC AND NUCLEOTIDE EDITING PATHWAYS ASSOCIATED WITH *MYCOBACTERIUM TUBERCULOSIS* COMPLEX (MTBC) LINEAGE

Theophilus Afum[1], Prince Asare[1], Stephen Osei-Wusu[1], Britta S. Meyer[2], Tobias L. Lenz[2], Dorothy Yeboah-Manu[1]

[1]Noguchi Memorial Institute for Medical Research, University of Ghana, Accra, Ghana, [2]Research Unit for Evolutionary Immunogenomics, Department of Biology, University of Hamburg, Hamburg, Germany

Tuberculosis (TB) susceptibility is shaped by complex interactions between host genetic factors and genetically diverse members of the *Mycobacterium tuberculosis* complex (MTBC). The *Mycobacterium africanum* (Maf), which is largely restricted to West Africa, differs epidemiologically from the *M. tuberculosis* sensu stricto (Mtbss); however, the host genetic architecture underlying lineage-specific susceptibility remains poorly understood. Here, we performed a host genetic analysis in a Ghanaian cohort of 232 TB cases (70 Maf and 162 Mtbss-Lineage_4 infected), integrating population structure assessment, genome-wide single-variant association testing, and gene- and pathway-level enrichment analyses. Our findings showed no evidence of strong population stratification in the Ghanaian population, in both host genetic variation and infecting MTBC lineage. This allowed for focusing on specific host-pathogen interactions without confounding population structure. No single nucleotide polymorphism (SNP) reached genome-wide significance; however, suggestive association signals, rs57977159 and rs1509160, were observed at loci *RASGEF1B* and *B9D1*. Gene-based analysis identified *B9D1* as significantly associated after Bonferroni correction (2.01×10^{-6}). Pathway enrichment analysis identified overrepresentation of immune regulatory processes, including purinergic nucleotide receptor activity, deoxycytidine deaminase activity, and G-protein-coupled purinergic nucleotide receptor activity. Together, these findings suggest that lineage-specific TB susceptibility may be influenced by how the host calibrates immune activation, rather than immune strength alone, highlighting potential targets for host-directed interventions.

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LEPROSY STIGMA IN ZOU DEPARTMENT OF BENIN: MAGNITUDE AND ASSOCIATED FACTORS

Sètondji Diane Zanvo[1], Christelle Gbaguidi[1], Armelle Hounkpatin[2], Jean Gabin Houezo[1], Inès Agbo[1], Cristina Juan Jimenez[3], Anna Gine-March[3], Pierre Velut[4], Sékou Samadoulougou[5], Christian Johnson[1]

[1]Université of Abomey – Calavi, Centre Interfacultaire de Formation et de Recherche en Environnement pour le Développement Durable (CIFRED), Abomey-Calavi, Benin, [2]Université Nationale des Sciences, Technologies, Ingénierie et Mathématiques, Abomey, Benin, [3]Anesvad Foundation, Bilbao, Spain, [4]Fondation Raoul Follereau, Paris, France, [5]Université libre de Bruxelles, Bruxelles, Belgium

Since ancient times, leprosy has been regarded as an incurable and contagious disease that causes deformities of the face, hands, and feet. These deformities are a major source of rejection, discrimination, and social exclusion for affected individuals. The objective of this study was to assess the magnitude of leprosyrelated stigma and its associated factors, distinguishing between selfstigma among persons affected by leprosy and perceived stigma among their contacts in Zou Department of Benin. This was a crosssectional, descriptive, and analytical study conducted from 29 October 2024 to 28 February 2025. It included former multibacillary leprosy patients (PAL) registered between 2013 and 2023 in Zou Department in southern Benin, as well as their close contacts living within a 100meter radius. Data were collected using the ODK Collect (opensource mobile data collection tool). The standardized Explanatory Model Interview Catalogue was used to assess stigma. Given the asymmetric distribution, associations between explanatory variables and stigma scores were examined using nonparametric tests (Mann–Whitney and Kruskal–Wallis). Quantile regression was used to identify associated factors. A total of 92 PAL and 343 close contacts were included in the analysis. The median total stigma score was 13.5 (IQR: 17.5) among PAL and 18 (IQR: 10.5) among contacts. Furthermore, the results show that both the mode and timeliness of case detection significantly influenced selfstigma. Compared with passively detected patients, those detected actively had significantly higher median selfstigma scores ($p = 0.001$). Late detection was also associated with increased selfstigma ($p = 0.003$) compared with early detection. Among close contacts, individuals residing in rural areas exhibited higher median stigma scores ($p = 0.034$). Leprosy related stigma remains high in Zou Department, with disparities according to place of residence, suggesting the influence of socio-cultural factors. Targeted community-based interventions, adapted to local contexts, are needed to correct misconceptions and improve attitudes toward leprosy.

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URINARY TRACT INFECTION IN TYPE II DIABETIC PATIENTS

Alberta Kokoi Akyereku Tackie

Noguchi Memorial Institute for Medical Research, Legon, Ghana

Urinary tract infection (UTI) is one of the most common bacterial infections affecting individuals worldwide, and its incidence is notably higher among patients with diabetes mellitus, particularly those with Type 2 diabetes. This study was conducted at Korle Bu Teaching Hospital to assess the prevalence and nature of urinary tract infections among Type 2 diabetic patients. A total of 100urine and blood samples were collected from diabetic patients for microbiological and biochemical analysis. The urine samples were cultured and subjected to various biochemical tests including indole, citrate utilization, urease, triple sugar iron agar, and cytochrome oxidase tests for microbial identification.The results revealed that UTIs were more prevalent among females (54%) than males (21%), indicating that gender plays a significant role in susceptibility, likely due to anatomical differences. The most frequently isolated pathogen was Escherichia coli (35%), followed by Pseudomonas species (15%) and Enterobacter species (15%). Other isolated organisms included Proteus species, Streptococcus faecalis, and Klebsiella species. The age group 61-70 years demonstrated the highest prevalence of infection, with an overall mean age of 67.94 years, suggesting that older Type 2 diabetic patients are at greater risk of developing UTIs.The study concludes that there is a strong association between Type 2 diabetes and urinary tract infections, with E. coli being the predominant causative organism. Poor glycemic control, bladder dysfunction caused by diabetic neuropathy, and impaired immune response are major contributing factors. Based on these findings, it is recommended that health education on diabetes management, infection prevention, and regular medical screening be intensified to reduce UTI incidence and prevent renal complications among diabetic patients.

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CELLULAR TROPISM AND LIGAND SPECIFICITY OF TICK-BORNE *ANAPLASMA PHAGOCYTOPHILUM* IN HUMAN MYELOID AND ENDOTHELIAL CELLS

Sabarinath Neerukonda[1], Vedha Paidipally[1], Nirmallya Acharyya[1], kazuyo takeda[1], Anne eder[1], Sanjai Kumar[1], Brendan Elsworth[2]

[1]FDA/CBER/OBRR, Silver Spring, MD, United States, [2]FDA/CBER/OBRR, Silver spring, MD, United States

Anaplasma phagocytophilum (*Ap*) is an obligate intracellular gram-negative bacterium that can be transmitted by ticks or via blood transfusion to cause Human Granulocytic Anaplasmosis (HGA). In humans, *Ap* infects and replicates in neutrophils, bone marrow progenitors, and endothelial cells. To study *Ap* host cell tropism and biomarkers of infection, we compared four myeloid (HL60, K562, U937 and THP1) and five endothelial (RF/6A, HBMEC and HUV-EC-C, HUVEC/Tert2, TIME) cell lines for their ability to support *Ap* replication. Cell lines were infected with cell-free *Ap* and the counts of infected cells and number of bacterial genomes were quantified over a four day period by microscopy and quantitative real time PCR. In addition to HL60, a promyelocytic leukemia cell line that is widely used for *Ap* propagation, we find that K562, U937 and THP1 cells support *Ap* replication. K562 is an erythroleukemia cell line that can spontaneously differentiate into early precursors of the monocytic, granulocytic, and erythroid series. Our results demonstrate that K562 may serve as an additional model to study *Ap*-infection of myeloid progenitors and U937 and THP1 cell lines may model *Ap*-infection of monocytic precursors. Except HBMEC, remaining four endothelial cell lines supported *Ap* replication. We investigated known entry mechanisms across host cells and found that all myeloid cells were sialic acid- and PSGL-1 dependent, whereas there were differences between endothelial cells. Together, these data demonstrate significant differences in host cell susceptibility to *Ap* infection and provide improved models for the study of *Ap* pathogenesis and host biomarkers across different tissues.

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LEPROSY BEYOND HUMAN HOSTS: A ONE HEALTH SYSTEMATIC REVIEW

Nithya Narayanaswamy[1], Tolulupe Ojo-akosile[1], Kayla Shore[1], Jessica K. Fairley[2]

[1]Rollins School of Public Health, Emory University, Atlanta, GA, United States, [2]Emory University, Atlanta, GA, United States

Despite global elimination targets, Hansen's disease (leprosy) persists endemically across tropical and subtropical regions, with pockets of hyperendemicity. Transmission dynamics extend beyond person-to-person contact, and growing evidence implicates environmental reservoirs, WASH conditions, climate variability, and zoonotic pathways in the persistence of *M. leprae.* We conducted a systematic review to characterize the contribution of environmental pathways to leprosy transmission and persistence. The PECOS (Population, Exposure, Comparison, Outcome, and Study type) framework guided the design and scope of the study, prioritizing individuals infected with or exposed to *M. leprae*, as well as populations at risk due to environmental, WASH-related, zoonotic, or human-wildlife and human-environment interactions relevant to leprosy incidence or endemicity. Thematic analysis identified zoonotic transmission via armadillos as a consistent non-human pathway, with genetic similarity between animal and human strains documented regionally across Brazil and the southern United States. Additionally, water bodies may serve as plausible environmental reservoirs, as *M. leprae* has been detected in surface water, though a direct link with case emergence remains inconclusive. Social determinants including poverty, overcrowding, and poor water access operate as risk multipliers. Climate and seasonality remain the least studied, with preliminary evidence linking monsoon conditions to increased *M. leprae* detection. Evidence supports an eco-epidemiological model in which zoonotic, environmental, and social pathways interact to sustain transmission. These findings highlight the importance of integrated environmental surveillance and One Health approaches for leprosy. They further provide a framework for early diagnosis, healthcare allocation, and disability prevention that may also be applied to other environmentally transmitted neglected tropical diseases.

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DETERMINATION OF THE GENETIC DIVERSITY OF PATHOGENIC LEPTOSPIRA SPECIES IN BATS CAPTURED IN THE DEPARTMENTS OF CÓRDOBA AND SUCRE, COLOMBIA

Virginia C. Rodriguez-Rodríguez, Alfonso Calderón-Rangel, Ana M. Castro-Cordero, Camilo A. Guzmán -Teran, Valeria Izquierdo-Agamez

Universidad de Córdoba, Monteria, Colombia

Leptospirosis is a neglected zoonosis with a high incidence in both tropical and subtropical regions, which have social and environmental characteristics that favour the maintenance of pathogenic Leptospira species in the environment. Bat species inhabit a wide range of environments worldwide and form large colonies, which play a crucial role in the persistent circulation of pathogens. Human activities that encroach on bat habitats and increase urbanization, bringing bats closer to humans, likely increase the opportunities for the spread of Leptospira spp. In Colombia, the wide geographic distribution of bats, their ability to adapt to diverse ecosystems, and their constant interaction with other animals and the environment make them potential reservoirs of Leptospira spp. The objective was to determine the genetic diversity of pathogenic Leptospira species in bats captured in the departments of Córdoba and Sucre, Colombia. An observational analytical study was conducted. Using simple random sampling, kidney samples were selected from 259 bats captured in rural areas of the departments of Córdoba and Sucre. The presence of Leptospira spp. was detected by PCR; 41 kidneys were positive. Twenty-six samples were analyzed by MLST using the scheme proposed by Ahmed for six loci: adK, icdA, lipL32, lipL41, rrS2, and secY. Natural infection was demonstrated in bats from the departments of Córdoba

and Sucre with five Leptospira species: Leptospira borgpetersenii (n=15, 57.7%), Leptospira kirschneri (n=5, 19.2%), Leptospira alexanderi (n=3, 11.5%), Leptospira noguchii (n=2, 7.7%), Leptospira weilii (n=1, 3.8%), and the Javanica, Autumnalis, and Sejroe serogroups. These findings highlight the fundamental role of bats in the maintenance and transmission cycles of *Leptospira* spp.

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DETERMINATION OF PATHOGENIC *LEPTOSPIRA* SPECIES IN BUFFALOES OF THE UPPER SINÚ REGION OF CÓRDOBA, COLOMBIA

Virginia C. Rodriguez-Rodríguez, Alfonso Calderón-Rangel, Ana M. Castro-Cordero, Daniela Correa-Camelo, Leidy L. Ortiz-Charrasquiel

Universidad de Córdoba, Monteria, Colombia

Leptospirosis is a zoonosis with a global distribution, whose health and economic impact makes it a priority problem for public health and animal health. Transmission to humans occurs mainly through contact with the urine of infected animals or with contaminated environments. Despite the growth of the buffalo sector in Córdoba, information on the epidemiological role of buffalo in the transmission cycle remains limited. The objective was to determine the pathogenic *Leptospira* spp. species present in buffaloes from the department of Córdoba. A cross-sectional observational study was conducted to characterize the pathogenic Leptospira spp. species present in buffaloes from the department of Córdoba. Simple random sampling was performed, through which 272 serum samples were taken and subjected to Microscopic Agglutination Test (MAT) using a panel of 16 serogroups. To identify renal carriers, urine samples were collected from ten animals selected based on their serological titers. These samples were analyzed by culture on EMJH medium and nested PCR targeting the 16S rRNA gene. PCR-positive samples were then subjected to MLST using the scheme proposed by Ahmed for six loci: adK, icdA, lipL32, lipL41, rrS2, and secY. The overall seroprevalence of Leptospira spp. was 72.4%. The predominant serogroup was Grippotyphosa (47.8%), followed by Australis (5.9%) and Tarassovi (2.9%). Mixed serological patterns were identified in 5.1% of the animals. Renal carriers were detected by PCR in four of the ten samples analyzed; two of these samples corresponded to *L. borgpetersenii* serogroup Pomona *and L. borgpetersenii* serogroup Ballum. The results demonstrate widespread circulation of pathogenic Leptospira spp. serogroups and confirm the presence of renal carriers in buffalo in Córdoba, highlighting their role in the eco-epidemiological cycle of leptospirosis. From a One Health perspective, these findings underscore the need to strengthen integrated surveillance and prevention strategies in tropical production systems.

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IDENTIFYING RISK FACTORS FOR LEPROSY INFECTION AND DISEASE RELATED TO UNSAFE WASH (WATER, SANITATION AND HYGIENE) AND ENVIRONMENTAL EXPOSURES IN BRAZIL

Anisa Carolyn Iqbal[1], Nithya Narayanaswamy[1], Audra Bass[1], Erica B. M. Silva[2], Maisa Pereira Vieira[2], Daisy Monteiro[2], Marcos D. S. Pinheiro[2], Pedro Marcal[3], Lorena Bruna Pereira de Oliveira[4], Alexndre Branco[5], Lucia Alves de Oliveira Fraga[6], Jessica K. Fairley[1]

[1]Emory University, Atlanta, GA, United States, [2]Universidade Federal de Juiz de Fora - GV, Governador Valadares, Brazil, [3]Georgia Institute of Technology, Atlanta, GA, United States, [4]UNIVALE, Governador Valadares, Brazil, [5]Creden-PES, Governador Valadares, Brazil, [6]Universidade Federal de Juiz de Fora - Campus GV, Governador Valadares, MG, Brazil

Recent evidence has shown that environmental and zoonotic reservoirs of *Mycobacterium leprae* may contribute to leprosy transmission. To further this research, we investigated associations between WASH (water, sanitation and hygiene) or environmental factors and both *M. leprae* seropositivity and the development of clinical disease in infected individuals. We hypothesized that contact with possible environmental reservoirs may lead to both increased seropositivity as well as development of leprosy in already infected individuals. We recruited 1,315 participants in 4 municipalities of Minas Gerais, Brazil, and used the LID-1 and PGL-1 antigen tests as proxies for exposure to *Mycobacterium leprae* and increased susceptibility to Hansen's disease. Demographics, WASH factors and environmental factors were collected through surveys. A second cohort of 478 participants was then recruited and tested for anti-PGL-I IgG, with plans to also measure anti-LID-1. Descriptive, chi-square analyses were undertaken. In the first cohort, 19 (out of 79) anti-LID-1 positive participants developed Hansen's disease during the 3-year study and 29 seropositive, asymptomatic controls were analyzed against them. Leprosy was associated with a lower odds of rural residence (OR 0.13, 95% CI 0.01-0.89), which was a disparate finding from our previous research. In addition, low income and lack of regular handwashing trended towards significance, while other environmental factors did not show associations. For the second cohort using anti-PGL-I IgM, 39 (8.2%) tested positive, a high percentage in a random community sample. Seropositive individuals were more likely to be under 15 years old (OR 3.44, 95% CI 1,20, 9.84), a surprising finding. Low SES (OR 1.93, CI 0.91-3.97), produce washed with water only (OR 3.43, CI 0.93-10.57) showed strong associations while not statistically significant. While many did not reach statistical significance, our results show demographic and potential environmental factors associated with leprosy infection and development of disease that should be further studied in adjusted analyses and higher powered samples.

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HOST NEUTROPHIL DYSFUNCTION IN ACUTE MELIOIDOSIS PATIENTS WITH AND WITHOUT DIABETES

Sophana Chea[1], Sreyngim Lay[2], Sreytouch Chea[1], Somnang Man[2], Piseth Ly[1], Sokna Ly[1], Pich Chea[1], Chiny Voeurn[1], Ratanak Sath[1], Denis Laurent[3], Kimsreng Lean[4], Chanty Ny[5], Vantha Te[6], Kosal Thay[7], Heng Seng[8], Sovann Ly[8], Heather Teague[9], Jeffrey Strich[9], Chanthap Lon[1], Michail Lionakis[10], Christina Yek[1]

[1]International Center of Excellence in Research, National Institute of Allergy and Infectious Disease, National Institutes of Health, Phnom Penh, Cambodia, [2]National Center for Parasitology, Entomology, and Malaria Control, Ministry of Health, Phnom Penh, Cambodia, [3]Kantha Bopha Hospital, Phnom Penh, Cambodia, [4]National Pediatric Hospital, Phnom Penh, Cambodia, [5]Cambodia-China friendship Preah Kossamak Hospital, Phnom Penh, Cambodia, [6]Takeo Provincial Hospital, Phnom Penh, Cambodia, [7]Khmer-Soviet friendship Hospital, Phnom Penh, Cambodia, [8]Communicable Disease Control Department, Ministry of Health, Phnom Penh, Cambodia, [9]Laboratory of Critical Illness Pathogenesis and Therapeutics, National Heart Lung and Blood Institute, National Institutes of Health, Bethesda, MD, United States, [10]Laboratory of Clinical Immunology and Microbiology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bathesda, MD, United States

Melioidosis, caused by *Burkholderia pseudomallei*, is strongly linked to diabetes. Polymorphonuclear leukocytes (PMNs) are key to host defense, and diabetes-related dysfunction may increase susceptibility. We compared PMN phenotype and function in hospitalized patients with melioidosis (with and without diabetes), non-melioidosis sepsis (NMS), and afebrile controls. Since October 2025, 59 melioidosis cases, 21 NMS cases, and 47 controls were enrolled at six Cambodian hospitals. Whole blood was stained for CD45, a viability and lineage exclusion markers, and PMN markers including CD15, CD10, CD16, CD18, CD33, CD11a, CD11b, CD62L, CD63, and intracellular MPO. Oxidative burst was assessed by dihydrorhodamine-123 (DHR) oxidation after PMA stimulation. Phagocytosis was measured in PMNs by negative immunomagnetic selection using fluorescent *E. coli* bioparticles. The proportion of CD15+ PMNs among CD45+ leukocytes was similar across groups. Compared with controls, melioidosis cases had more immature and activated PMNs, with lower CD10 ($p=0.01$), higher CD63 ($p=0.03$), and lower CD16 expression ($p<0.01$). PMN phenotypic markers did not differ significantly between diabetic (N=9) and non-diabetic melioidosis participants (N=8). Oxidative burst was reduced in both melioidosis and NMS patients versus controls (both $p<0.01$) and was lower among melioidosis patients with diabetes compared to non-diabetic participants (N=28 each; $p=0.02$). Phagocytic activity was also reduced in melioidosis and NMS ($p=0.01$ and $p=0.0002$, respectively), with lower

activity in NMS than in melioidosis (p=0.02). Among melioidosis patients, phagocytic activity did not differ significantly between diabetic and non-diabetic participants (N=23 and N=15). Taken together, we found PMN dysfunction, with reduced oxidative burst and phagocytosis, in patients with melioidosis and NMS. In this preliminary analysis with a small number of melioidosis patients, oxidative burst was reduced in diabetic versus non-diabetic melioidosis cases. Further studies are needed to define specific PMN defects that contribute to susceptibility to melioidosis.

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PREDICTING INVASIVE NONTYPHOIDAL SALMONELLA DISEASE USING ROUTINE INFORMATION COLLECTED IN CLINICAL SETTINGS IN A SLUM POPULATION IN NAIROBI

Kelvin Kimutai Kering, Cecilia Mbae, Samuel Kariuki
Kenya Medical Research Institute, Nairobi, Kenya

Invasive Nontyphoidal Salmonella (iNTS) disease is a major cause of morbidity and mortality in sub-Saharan Africa. Accurate clinical diagnosis in settings lacking microbiological capacity, could improve patient outcomes. This study sought to identify clinical, demographic, WaSH and environmental factors that, when evaluated together, improve empiric prediction of iNTS among febrile patients presenting at treatment centers in Nairobi's informal settlements. This analysis utilized data from a study in Mukuru slums in Nairobi, Kenya, between 2013 and 2018, involving individuals aged ≤16 years who presented to clinics with fever. Clinical, demographic, household WaSH and environmental factors collected at presentation were dichotomized to "favorable" (lower risk of iNTS) or "non-favorable" (higher risk of iNTS). The data were further divided into two groups: children < 5 years and ≥ 5 years. Using a decision tree created by recursive partitioning, machine learning algorithm, we developed dichotomous composite variables predictive of blood culture-confirmed iNTS. An initial composite variable was developed using selected socio-demographic and clinical features (model 1). A second variable was developed using clinical, sociodemographic, household WaSH and environmental factors (model 2). In children <5 years, model 1 identified any malnourished child with an axillary body temperature ≥38°C, all children with axillary temperatures ≥38°C who were aged 2-4 years, and children with axillary temperatures <38°C without diarrhea as being predictive of iNTS (sensitivity 77%, specificity 49%; area under the receiver operating characteristic (ROC) curve 0.65, 95%CI: 0.64-0.67). Model 2 did not improve the prediction in this age group, and only an axillary temperature of ≥38°C was predictive of iNTS for children ≥5 years (sensitivity, specificity and AUC 79%, 37% and 0.58, respectively). The composite variable composed of clinical and socio-demographic variables was predictive of iNTS disease in patients <5 years. Addition of WaSH and environmental factors did not improve iNTS prediction.

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RAPID TRACHOMA ASSESSMENTS IN VULNERABLE AMAZONIAN DISTRICTS OF ECUADOR: EVIDENCE OF FOCAL TRANSMISSION AND IMPLICATIONS FOR ELIMINATION

Valeria Patricia Torres Espín
UTE University, Quito, Ecuador

Trachoma, the leading infectious cause of preventable blindness, has declined markedly in Latin America, recent epidemiological data from Ecuador remain limited. This study aimed to assess the presence and epidemiological characteristics of trachoma in highly vulnerable rural and Indigenous communities of two Amazonian departments using rapid trachoma assessments. A point prevalence survey was conducted in selected parishes identified through a vulnerability index including limited access to water and sanitation, high poverty and illiteracy rates, and proximity to historically endemic areas. Household visits included key informant interviews and standardized eye examinations following WHO grading criteria. Children aged 1-9 years were examined for trachomatous inflammation-follicular and individuals aged ≥15 years for trachomatous trichiasis. Data were collected using paper forms and digitized in a Tropical Data-compatible platform. A total of 1,824 individuals were examined, of whom 19.8% were children aged 1-9 years. These findings demonstrate active focal transmission in Amazonian Ecuador, primarily affecting children, and support the urgent implementation of the SAFE strategy, inclusion of these areas in national elimination plans, and strengthened surveillance and water, sanitation, and hygiene interventions.

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OPTIMIZATION OF ANTIGEN PRODUCTION FOR *STRONGYLOIDES STERCORALIS* RAPID TEST PLUS (SSRT+), A SEROLOGICAL RAPID DIAGNOSTIC TEST IN DEVELOPMENT FOR THE SURVEILLANCE OF STRONGYLOIDIASIS WORLDWIDE

Mykelti O'Brien[1], Justin Nueve[1], Daniela Oltean[1], Jean Saunders[2], Rahmah Noordin[3], Tomoaki Taniguchi[4], Tomoaki Uchiki[4], Junichi Akatsuka[4], Matthias Schwarz[2], Marco Biamonte[2]
[1]Big Eye Diagnostics, San Diego, CA, United States, [2]Drugs & Diagnostics for Tropical Diseases (DDTD), San Diego, CA, United States, [3]Universiti Kebangsaan Malaysia (UKM), Kuala Lumpur, Malaysia, [4]Medical and Biological Laboratories Co., Ltd, Tokyo, Japan

Strongyloidiasis is caused by *Strongyloides stercoralis* and affects over 600 million people globally, largely in tropical and subtropical regions. SsRapid is a serological point-of-care test utilizing recombinant NIE protein, with laboratory and clinical sensitivity and specificity values in the range of 80-97% and 90-100%, respectively. SsRapid is formatted as a two-way test requiring 35 µL of sample, which limits ease of use and complicates manufacturing. DDTD is leading a collaborative effort to develop a low-cost, serological diagnostic test that meets all key criteria of the WHO draft strongyloidiasis TPP and is suitable for large-scale deployment in endemic settings. To that end, SsRapid is being converted to a one-way IgG4 capture format, reducing the required volume of sample by 14x, in turn simplifying testing workflow and manufacturing. A key component of this development was optimizing the Ss-NIE antigen, which has historically faced protein expression and stability issues, raising concerns about manufacturing consistency at scale. Predicted immunogenic epitopes are localized in NIE domain 2, but the recombinant protein typically used for rapid diagnostics is comprised of domains 1 and 2. To test whether domain 1 contributes to expression/stability issues without adding diagnostic value, NIE antigen variants, comprised of both domains as well as domain 2 alone, were produced using various N-terminal and C-terminal affinity tags in *E. coli*. Depending on the purification tag, assay specificity ranged from 48-100% (n=40) and sensitivity ranged from 33-100% (n=6), guiding antigen selection for optimization. To further improve antigen stability post-purification, the top NIE variant was evaluated in various dialysis buffers. Assay sensitivity ranged from 82-100% (n=22) and specificity ranged from 75-90% (n=20), depending on the buffer formulation. These findings highlight the importance of optimizing protein expression in addition to assay optimization to achieve high-performing diagnostics. A lead antigen-buffer combination has been identified, and full lateral flow assay optimization is ongoing.

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UNCOVERING ANTIBODY RESPONSE TO A HUMAN HOOKWORM VACCINE: INSIGHTS FROM CLINICAL AND CHALLENGE STUDIES

Jessica Lynn Dade[1], Camila Queiroz-Glauss[1], Maria Elena Bottazzi[2], Peter Hotez[2], Caroline DiRosato[1], Jeffrey Bethony[3], David Joseph Diemert[3], Pedro Henrique Gazzinelli-Guimaraes[4]
[1]George Washington University, Washington, DC, VA, United States, [2]Baylor College of Medicine, Houston, TX, United States, [3]George Washington University, Washington, DC, United States, [4]The George Washington University, Washington, DC, United States

Recently, a human hookworm vaccine, based on the Na-GST-1 antigen adsorbed on Alhydrogel and co-administered in the absence or presence of a Toll-like receptor 9 agonist (CpG) was developed and shown to provide

relevant protection against challenge infection in naive adults participating in a phase 2 randomized controlled trial. We evaluated *Na*-GST-1-specific antibody responses during and after a three-dose vaccination regimen in the trial participants followed by controlled *Necator americanus* infection. Plasma samples collected longitudinally were analyzed by indirect ELISA. *Na*-GST-1-specific total IgG and IgG3 demonstrated two notable peaks following the second and third immunizations, the latter representing the maximal response. This pattern was observed for both *Na*-GST-1/Alhydrogel and *Na*-GST-1/Alhydrogel /CpG-adjuvanted formulations. The addition of CpG 10104 further enhanced IgG1 responses, which mirrored overall IgG kinetics, whereas Alhydrogel alone elicited comparatively lower magnitude responses. Post-vaccination and up to 90 days post-challenge infection, there was a significant and sustained increase in Na-GST-1-specific IgG levels in the group that received the CpG-adjuvanted formulation compared to the Alhydrogel-only group. Administration of *Na*-GST-1 with Alhydrogel alone showed that three doses are necessary for an elevation in IgM and IgG4 antibodies in circulation. In most volunteers, CpG addition did not appear to change IgM and IgG4 levels over the full course. Preliminary analyses suggest that a substantial proportion of the IgG response may be under IgG1-driven mechanisms, while IgE responses remained negligible throughout vaccination and post-challenge. Future studies incorporating affinity measurements and IgD/IgA antibody quantification will be critical to further define the functional quality of vaccine-induced immunity. These data suggest that CpG enhances the magnitude and durability of IgG subclass responses, whereas Alhydrogel alone elicits lower and less sustained antibody levels, highlighting distinct humoral profiles induced by each formulation.

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VALIDATION OF REACTIVITY OF IMMUNOGLOBULINS IN POOLED DRIED BLOOD SPOTS FOR DETECTION OF OV16 ANTIGEN

Camila Trejo Paz[1], Makayla Miley[2], Hassan Hassan[1], Thomas R. Unnasch[3], Kristi Miley[1]

[1]University of South Florida, Tampa, FL, United States, [2]Pasco Hernando State College, Brooksville, FL, United States, [3]Tampa, FL, United States

Onchocerciasis remains endemic in two main areas of the world, Latin America and Africa, where 250 million people are at risk of infection. Despite the World Health Organization's many prevention, control, and surveillance programmes, this ocular and skin disease has proven to be hard to eradicate in these resource-limited zones. The gold standard for Onchocerciasis diagnostics is skin snip microscopy. Current treatment involves mass drug administration of ivermectin, which significantly decreases the microfilarial load in the skin and drastically reduces the sensitivity of the test in treated populations. As a result, enzyme-Linked Immunosorbent Assays (ELISAs) with disease specific antigens are recommended by the WHO to aid in surveillance efforts. However, these assays are time costly and pose a challenge in under-resourced endemic areas. This research demonstrates a methods-based approach to improve testing capacity for surveillance of onchocerciasis, especially in areas that are resource limited. The approach evaluated pooled dried blood spots (DBS) for testing, allowing for multiple DBS to be tested together as an effective alternative to testing all DBS individually. As Onchocerciasis endemic regions begin to reach elimination, it becomes difficult to detect positives within the population at large. This research evaluates a pooled DBS testing method using both the Ov16 ELISA and the Ov16 Onchocerciasis Rapid Diagnostic Test (RDT) platforms. Results demonstrated that Pooled DBS testing has the capacity to detect one positive DBS in a pool of known negative DBS with precision and accuracy. Pooled DBS testing is a promising alternative for optimizing laboratory personnel and supplies, especially in endemic areas where resources may be limited.

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A CROSS-SECTIONAL STUDY TO ASSESS FILARIAL INFECTION AMONG THE NEVER TREATED INDIVIDUALS IN SELECTED DISTRICTS OF INDIA

Dinesh Raja J[1], Srividya Adinarayanan[1], Muhammed Jabir MM[1], Anju Viswan K[2], Kishanthini Manikandan[1], Srimathi K[1], Rinku Sharma[3], Tanu Jain[3], Manju Rahi[1]

[1]ICMR Vector Control Research Centre, Puducherry, India, [2]ICMR Vector Control Research Centre, Koraput, India, [3]National Center for Vector Borne Diseases Control, New Delhi, India

With the global lymphatic filariasis (LF) elimination goal set to 2030, one of the most critical programmatic challenges, are the never treated individuals defined as those who self-report having never taken antifilarial drugs during any mass drug administration (MDA) rounds. These individuals could act as potential reservoirs of infection, aiding transmission. Hence, a study is underway to estimate the prevalence of filarial infection among the never treated individuals (defined as never treated for at least five consecutive rounds) and identify the factors and barriers impeding their access and participation in MDA, using a mixed method approach in four LF endemic districts of India. A multi-stage cluster sampling design is followed to select 30 Health sub centres from one highly endemic block in each of the districts. A minimum sample size of 2535 never treated individuals per block are being assessed for the circulating filarial antigenemia (CFA) and microfilaremia (Mf) through cross-sectional blood surveys. Knowledge, attitude and practice (KAP) surveys, in depth interviews and focus group discussions are being conducted to elicit the reasons for their non-compliance. Preliminary results from 2 of the 4 blocks, covering 5326 never treated individuals (57.2% males) found a high CFA prevalence of 19.4% (95%CI 18.3-20.5), and was significantly higher in males 22.9% (21.5-24.5) compared to females 14.6% (13.2-16.1). The night blood smears collected from 941 CFA positive individuals are currently under examination. In depth analysis of infection parameters with that of KAP and qualitative surveys will help in identifying various reasons for non-participation. This will enable the programme to design tailored communication and community engagement strategies to bring them under the umbrella of MDA, strengthening progress towards LF elimination.

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EPIDEMIOLOGICAL ASSESSMENT OF LYMPHATIC FILARIASIS STATUS IN POST MASS DRUG ADMINISTRATION AREAS AND RISK OF TRANSMISSION IN THE CONTEXT OF MIGRANTS IN INDIA

Srividya Adinarayanan[1], Dinesh Raja J[1], Jabir Muhammed MM[1], Anju Viswan K[1], Balasubramaniyan Ramalingam[1], Manikandan Kishanthini[1], Srimathi K[1], Rinku Sharma[2], Tanu Jain[2], Manju Rahi[1]

[1]ICMR-Vector Control Research Centre, Puducherry, India, [2]National Center for Vector Borne Diseases Control, New Delhi, India

In India by 2025, 125 out of 345 (36.2%) lymphatic filariasis (LF) endemic districts had stopped Mass Drug Administration (MDA) and were under post MDA surveillance. As the current situation of LF is unknown in these areas, with increased mobility of population from the endemic districts to these areas, there is an imminent risk of resurgence. Hence, this cross sectional study is planned to assess the epidemiological situation of LF in terms of human and vector infection in 3 evaluation units (EUs) under post MDA phase among: (i) adults aged ≥20 years in general population (n=3150/EU), (ii) migrants (aged ≥2 years) from endemic areas (n=1000/EU) and (iii) vectors (n=7500/EU). In one MDA naive EU bordering endemic district, in addition to migrant and vector surveys, school children aged 9 to 14 years (n=480) will be tested. KAP and qualitative surveys are conducted among migrants to understand their awareness and needs regarding MDA. Preliminary findings from the selected EU in Puducherry, with last MDA in 2011, the CFA prevalence in the general population (n=3252) was 0.6% (95% CI 0.4-0.9) with no Mf positives, while among migrants (n=1060), CFA and Mf prevalence were 12% (10.1-14.1) and 2.3% (1.5-3.4) respectively. A total of 13077 gravid female *Culex quinquefasciatus* were collected to

process for vector infection. In the selected EU in Surat district, where the last MDA was in 2016, of the total 3198 adults (48.1% males) tested, 6.3% (5.5-7.2) were CFA positive and in migrants (n=1000), it was 8% (6.4-9.9). Slide examination for Mf is under progress. While one EU demonstrated sustenance of transmission interruption (CFA<1% and Mf=0%), the other indicated ongoing transmission. However, in migrants, both EUs recorded high CFA and Mf positivity, indicating the need for testing migrants and understanding their role in the resurgence of LF in post MDA areas.

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PREVALENCE AND CLINICAL MANIFESTATIONS OF LOIASIS IN CHILDREN AND ADOLESCENTS IN RURAL GABON

Sarah Miebach[1], Rella Zoleko-Manego[2], Teite Hildebrandt[1], Sabine Belard[2], Ghyslain Mombo-Ngoma[2], Johannes Mischlinger[1], **Pia M. Michelitsch**[1], Maximilian Rakotonirinalalao[1], Michael Ramharter[1], Saskia D. Davi[1]

[1]Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany, [2]Centre de Recherches Medicales de Lambarene, Lambarene, Gabon

Loiasis is a neglected tropical disease caused by the filarial nematode *Loa loa* and transmitted to humans by *Chrysops* deer flies. The clinical manifestations of loiasis in adults—including eye-worm passage—are well documented. However, systematic data on the prevalence of loiasis and its clinical manifestations in children and adolescents are lacking. This study aims to determine the prevalence of loiasis in children and adolescents and to describe its clinical manifestations. Since May 2025, a cross-sectional survey has been conducted in the catchment area of the Centre de Recherches Médicales de Lambaréné (CERMEL) in rural Gabon. The study includes participants aged 1-15. The diagnostic criteria include detection of microfilariae in the blood as well as a WHO-established questionnaire assessing the symptom of eye worm migration. Clinical characteristics such as Calabar swellings, pruritus, and other non-specific symptoms are recorded using a standardized questionnaire. To date, 755 of 1000 children and adolescents have been enrolled. The median age is 7 years (IQR: 4-10). The study population includes 50.1% boys (378/755; 95% CI: 46.5-53.7%) and 49.9% girls (377/755; 95% CI: 46.3-53.5%). The overall prevalence of *Loa loa* was 9.9% (75/755; 95% CI: 7.8-12.1%). Further results, particularly regarding clinical manifestations, will be presented at the conference. These data place children and adolescents with loiasis into focus for the first time. In doing so, we highlight a previously overlooked population within an already neglected disease and argue that children and adolescents need to be explicitly included in prevention, diagnosis, and care programs.

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ONCHOCERCIASIS ELIMINATION MAPPING IN AKWA IBOM STATE, NIGERIA: INTEGRATED ENTOMOLOGICAL AND SEROLOGICAL EVIDENCE OF TRANSMISSION INTERRUPTION

Chukwuemeka Cletus Makata[1], Fatai Oyediran[1], Louise Makau-Barasa[2], Ellie Leaning[2], Kimberly Jill Kamara[3], Moses Oluwaseun Aderogba[4], Aliyu Muhammed[5], Kenneth N. Opara[6], Friday Maduka Chikezie[7], Clement Ameh Yaro[7]

[1]Federal Ministry Of Health, Nigeria, Abuja, Nigeria, [2]END Fund, New York, NY, United States, [3]The END Fund, Glen Head, NY, United States, [4]The END Fund, Lugbe, Nigeria, [5]Helen Keller, Abuja, Nigeria, [6]Univ of Uyo, Uyo PMB, Nigeria, [7]University of Uyo, Uyo, Nigeria

Onchocerciasis remains a major neglected tropical disease targeted for global elimination, yet historically hypoendemic or unmapped areas continue to pose risks of residual transmission. This study conducted Onchocerciasis Elimination Mapping (OEM) across Akwa Ibom State, Nigeria, to determine evidence of ongoing transmission using integrated entomological and serological approaches. A cross-sectional survey was implemented in 155 first-line communities across 31 Local Government Areas (LGAs). Entomological assessments involved prospection of 155 rivers and streams and human landing catches to detect adult *Simulium* vectors. Serological evaluation included 15,202 adults aged ≥20 years tested using Ov16 rapid diagnostic tests. No blackfly larvae or adult flies were detected across all surveyed sites, yielding an entomological positivity rate of 0% (95% CI: 0-2.4%). Similarly, all serological samples tested negative, corresponding to a prevalence of 0% (95% CI: 0-0.02%). Bayesian analysis assuming a Beta (1,1) prior produced a posterior distribution Beta(1, 15,203), with a mean prevalence of 0.0066% and a 95% credible interval of 0.00017%-0.0243%. The probability that prevalence exceeds 0.1% was effectively zero. These findings provide robust evidence of interrupted transmission of Onchocerca *volvulus* in Akwa Ibom State. However, given the ecological suitability of some river systems and proximity to endemic regions, sustained post-elimination surveillance is recommended.

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ESTIMATING THE PREVALENCE OF SOIL-TRANSMITTED HELMINTH (STH) AND SCHISTOSOMIASIS (SCH) INFECTIONS USING MODEL-BASED GEOSTATISTICS (MBG) IN OGUN STATE, NIGERIA

Tanaya Raj[1], Mark Minnery[2], Temitope Ogunbi[3], Imaobong Umah[4], Fatai Oyediran[4], Soneye Islamiat[5], Ima Chima[1], Ify Chime[3], Jerry Mbaka[3], Maryann Edeh[6], Gabriel Anwo[3], Vivian Batubo[3], Onyekachi Ifeoma[3], Ayoola Adegbile[3], Anam Anam Abdulla[7], Kate Elizabeth McCracken[8], Curtis Rodgers[1]

[1]Evidence Action, Washington, DC, United States, [2]Kelvin Grove, Australia, [3]Evidence Action, Abuja, Nigeria, [4]Federal Ministry of Health, Nigeria, Abuja, Nigeria, [5]Ogun State Ministry of Health, Abeokuta, Nigeria, [6]Lagos, Nigeria, [7]Evidence Action, Alexandria, VA, United States, [8]Durham, NC, United States

Nigeria, endemic for both soil-transmitted helminthiasis (STH) and schistosomiasis (SCH) infections, has administered routine preventative chemotherapy (PC) of school-aged children (SAC) in several states under the Federal Ministry of Health's deworming programme since 2017. After eight high-coverage rounds of PC in Ogun state, a cross-sectional, school-based, cluster survey of 2,154 SAC (8-14 years) across 41 schools was conducted to determine STH and SCH prevalence. A WHO-recommended model-based geostatistics (MBG) design was used to select survey sites and analyze data. MBG integrates baseline infection, ecological and environmental covariates, and treatment coverage to optimize site selection and estimate the probability that subnational units fall within programmatically relevant prevalence thresholds. Ogun State had a 0.996 probability of lying within 10-20% prevalence for any STH and a 0.998 probability of lying within 1-10% prevalence for any SCH. At a Local Government Area (LGA) level, the highest STH mean prevalence was estimated for Ogun Waterside (25.4%), and the lowest was estimated for Yewa South (5.8%). For SCH, the highest mean prevalence was estimated for Yewa North (7.7%) and Odeda (7.7%), while the lowest prevalence was estimated for Ijebu North East (0.3%). For any-STH infections, only light (172 pupils, 8%) and moderate (20 pupils, 0.9%) intensity infections were detected. For any-SCH infections, few heavy intensity infections were discovered (7 pupils, 0.3%) and the rest of the positive infections were light in intensity (30 pupils, 1.4%). The statewide relative reduction in prevalence from baseline was 63% for STH and 79% for SCH, indicating substantial progress potentially attributable to PC. Based on the 2025 prevalence classification, targeted LGA-level treatment for STH and ward-level treatment for SCH is recommended, in accordance with WHO guidelines.

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LEVERAGING THE HOST PLASMA PROTEOME TO DISTINGUISH FILARIAL INFECTION WITH *MANSONELLA PERSTANS, M. DEUX, AND LOA LOA*

Pinkus Tober-Lau[1], Clemens Dierks[1], Luzia Veletzky[2], Rella Zoleko-Manego[3], Ghyslain Mombo-Ngoma[3], Michael Ramharter[4], Markus Ralser[1], Florian Kurth[1]

[1]Charité - Universitätsmedizin Berlin, Berlin, Germany, [2]Medical University of Vienna, Department of Medicine I, Vienna, Austria, [3]Centre de Recherches Medicales de Lambarene, Lambarene, Gabon, [4]BNITM, Hamburg, Germany

Despite being the most prevalent filarial nematode in Africa with over 100 million people estimated to be infected, mansonellosis, caused by *Mansonella perstans* and the sympatric *M.Deux*, remains one of the most neglected tropical diseases. Consequently, significant knowledge gaps on basic biology and the impact on human host physiology remain. Furthermore, clinical diagnosis is complicated by overlapping symptoms with the co-endemic filariasis *Loa loa* and a lack of species-specific serology. We performed high-throughput mass spectrometry on plasma from 297 individuals in central Gabon: 93 with *Mansonella* monoinfection (10 *M. perstans*, 44 *M. Deux*, 27 both), 42 with microfilaraemic *L. loa*, 26 with *Mansonella* and *L. loa* co-infection, and 136 negative controls. Machine learning (ML) models were developed to identify and differentiate infection-specific molecular signatures. Twenty-one proteins were differentially abundant in *Mansonella* infections compared to negative controls. The host response was dominated by the humoral immune compartment, with higher abundances of immunoglobulins (e.g., IGHG3, IGHM), complement activators (C1QA-C), and the vascular adhesion molecule 1 (VCAM1). Notably, levels of IG(H)G3 and IG(H)M increased with age in infected individuals, suggesting progressing immune conditioning over time. *M. perstans* was associated with higher levels of cystatin c (CST3), beta-2-microglobulin (B2M), and adiponectin (ADIPOQ), compared to *M. Deux*. Applying a random forest based ML classifier, *Mansonella* could be distinguished from controls (AUROC 0.81) and from *L. loa* (AUROC 0.71). This study establishes a first plasma-proteomic fingerprint for mansonellosis, providing a molecular basis to distinguish between the closely related species *M. perstans* and *M. Deux*, and co-endemic *Loa loa*. In the absence of species-specific serology, these findings lay the groundwork for new diagnostic tools and a better understanding of the health burden of these highly neglected tropical filariases.

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EXPERIMENTAL IDENTIFICATION AND *IN SILICO* STRUCTURAL MODELING OF 3A-HSD IN *ONCHOCERCA VOLVULUS*: A POTENTIAL TARGET FOR ANTIFILARIAL DRUG REPURPOSING

Dennis Ofori Kyei[1], Prince Nyarko[1], John Asiedu Larbi[1], Prince-Charles Kudzordzi[2], Anabel Acheampong[3], Kenneth Bentum Otabil[4]

[1]Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, [2]Consortium for Neglected Tropical Diseases and One Health, University of Energy and Natural Resource, Sunyani, BA, Ghana, [3]Consortium for Neglected Tropical Disease and One Health, Sunyani, Ghana, [4]University of Energy and Natural Resources, Sunyani, Ghana

The search for alternative antifilarial therapies against *Onchocerca volvulus*, the causative agent of river blindness, is limited by insufficient knowledge of parasite metabolic targets. This study combined experimental and computational approaches to identify and characterize a predicted gene encoding 3-oxo-5-alpha-steroid IV dehydrogenase (3α-HSD) in adult *O. volvulus*, and to explore its relevance for drug repurposing.Genomic DNA was extracted from adult worms isolated from onchocercal nodules. Polymerase Chain Reaction (PCR) amplification was performed under optimized conditions, and products were visualized by gel electrophoresis. The amplified gene was sequenced using Sanger sequencing, confirming its presence and identity. Subsequent bioinformatics analyses were conducted using ExPASy for virtual translation and UniProt for functional annotation. A three-dimensional (3D) protein structure was predicted using SWISS-MODEL, and sequence homology was assessed using Clustal Omega. PCR and sequencing experimentally confirmed the presence of the 3α-HSD gene in *O. volvulus*. Computational analyses identified it as a 3-oxo-5-alpha steroid domain-containing protein, and structural modeling revealed a conserved 3D configuration comparable to homologous enzymes in other species, suggesting a functional role in parasite metabolism. This study provides the first experimental evidence and in silico structural insight into 3α-HSD in *O. volvulus* worms from Ghana, supporting its potential as a novel metabolic target for antifilarial drug repurposing. Further experimental validation of its function and druggability is warranted.

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MALARIA, SCHISTOSOMIASIS AND INTESTINAL HEALTH

Charlotte Willoughby

The University of Edinburgh, Edinburgh, United Kingdom

Background:Malaria and schistosomiasis remain major parasitic diseases, affecting approximately 240 million and 190 million individuals globally each year (WHO, 2021). Co-infections are common in Uganda and other regions of Sub-Saharan Africa, where interactions between *Plasmodium falciparum* and *Schistosoma mansoni* occur through modulation of the host immune system. Malaria induces a Th1-mediated pro-inflammatory response, whereas *S. mansoni* drives Th2 polarisation. These opposing pathways are mutually antagonistic, and Th2 dominance during co-infection may suppress Th1 activity, compromising malaria clearance and promoting intestinal inflammation through immune dysregulation. Methods: A cross-sectional field study of 400 participants was conducted across four Ugandan communities with varying *S. mansoni* endemicity. Blood and faecal samples were collected for both field and laboratory-based assays. Field diagnostics included malaria microscopy, two types of rapid diagnostic tests (HRP2/3- and LDH-based), haemoglobin quantification, Kato-Katz thick smears, and stool observations. Planned UK-based laboratory analyses include proteomics, quantitative PCR (qPCR), enteric pathogen screening, and inflammatory biomarker profiling. Results: Preliminary findings reveal that individuals with a co-infection of malaria and Schistosoma mansoni exhibit reduced haemoglobin levels and increased incidence of faecal occult blood and diarrhoea. Notable differences in prevalence of malaria were observed based on the diagnostic used, microscopy vs RDT, highlighting diagnostic challenges with a need for further confirmation and investigation into deletions of HRP2/3 genes via qPCR. In conclusion, the proposed conclusion and overall hypothesis of this study are that Malaria exacerbates intestinal inflammation during *Schistosoma mansoni* infection, driven by immune dysregulation and increased intestinal pathology due to malaria-associated inflammation and/or haemolytic anaemia.

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COLLAGEN IV NC1 DOMAIN PROTEIN: A CONSERVED TARGET FOR ADULTICIDAL THERAPY IN *ONCHOCERCA VOLVULUS*

Emmanuel Boateng Ansah[1], Prince-Charles Kudzordzi[2], Anabel Acheampong[3], Prince Nyarko[4], Kenneth Bentum Otabil[5]

[1]The Consortium for Neglected Tropical Diseases and One Health, Sunyani, Ghana, [2]Consortium for Neglected Tropical Diseases and One Health, University of Energy and Natural Resource, Sunyani, BA, Ghana, [3]Consortium for Neglected Tropical Disease and One Health, Sunyani, Ghana, [4]The Consortium for Neglected Tropical Diseases and One Health (NeTroDisOH), Sunyani, BA, Ghana, [5]University of Energy and Natural Resources, Sunyani, Ghana

Onchocerciasis (river blindness), caused by *Onchocerca volvulus*, affects over 37 million people worldwide, with more than 90 million at risk, predominantly in sub-Saharan Africa. Current control relies on mass administration of ivermectin, which effectively clears microfilariae but has limited activity against adult worms, hampering elimination efforts and raising concerns about emerging drug resistance. Identifying adulticidal targets is therefore critical for innovative therapeutic strategies.

Computational analyses have highlighted metabolic chokepoint enzymes as potential targets for repurposed drugs with adulticidal activity. Here, we experimentally validated the Collagen IV NC1 domain-containing protein (OVOC8585) in adult *O. volvulus*, confirming prior in silico predictions. Adult worms from endemic Ghanaian communities were analyzed using PCR with gene-specific primers, yielding the expected 421 bp fragment. Sanger sequencing produced 353 high-quality bases, showing 99% identity to the predicted gene. Three-dimensional structural modeling using SWISS-MODEL indicated a stable monomeric protein of 353 amino acids, with 92.2% sequence identity to *Onchocerca flexuosa*. Ramachandran analysis revealed 88.9% of residues in the core region, supporting structural integrity suitable for drug targeting. Multiple sequence alignment demonstrated strong conservation across filarial species, with 84–85% similarity to *Brugia pahangi*, *Wuchereria bancrofti*, and *Brugia malayi*. These findings establish OVOC8585 as a conserved, structurally stable protein critical for parasite survival. Its high conservation and structural suitability make it a promising candidate for repurposed adulticidal therapeutics, offering a potential complement to ivermectin in onchocerciasis elimination programs.

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TARGETING CALCINEURIN-LIKE PHOSPHOESTERASE IN *ONCHOCERCA VOLVULUS:* A PROMISING CANDIDATE FOR NEXT-GENERATION ANTIFILARIAL THERAPY

Prince Nyarko[1], John Asiedu Larbi[2], Prince-Charles Kudzordzi[3], Anabel Acheampong[4], Kenneth Bentum Otabil[1]

[1]The Consortium for Neglected Tropical Diseases and One Health (NeTroDisOH), Sunyani, Ghana, [2]Kwame Nkrumah University of Science and Technology, Kuamsi, Ghana, [3]Consortium for Neglected Tropical Diseases and One Health, University of Energy and Natural Resource, Sunyani, BA, Ghana, [4]Consortium for Neglected Tropical Disease and One Health, Sunyani, Ghana

Background: Onchocerciasis remains a major neglected tropical disease and the second leading infectious cause of blindness globally, with over 240 million people at risk, mainly in sub-Saharan Africa. Despite progress toward elimination, reliance on ivermectin is threatened by reports of reduced parasite susceptibility, highlighting the need for novel macrofilaricidal targets. Methods: Genomic DNA was extracted from four adult worms (n=4; two females, two males) isolated from nodules and amplified using optimized polymerase chain reaction protocols on a Proflex PCR system (Applied Biosystems, USA). Amplicons were visualized by agarose gel electrophoresis and sequenced using Sanger methods. Sequence quality was assessed, amino acid sequences derived using Expasy, and 3D structural models generated using SWISS-MODEL. Functional annotation was obtained from UniProt, and sequence similarity assessed using Clustal Omega. Results: The predicted CULP gene (OVOC11146) was confirmed in adult *Onchocerca volvulus*. Gel electrophoresis showed expected band sizes. BLASTn analysis revealed 99% identity in male isolates and 96% in female isolates relative to reference sequences. Structural modeling demonstrated conserved phosphoesterase domains associated with solute carrier family-like binding regions. Comparative analysis showed high similarity with related enzymes in *Onchocerca ochengi* (99.33%), *Parascaris univalens* (82.24%), and *Ascaris lumbricoides* (79.09%). This conservation suggests potential cross-species utility of targeted therapies. In conclusion, this study confirms the presence and sequence of the calcineurin-like phosphoesterase gene in *O. volvulus* and provides insight into its structural and functional properties. Its conservation across species supports its potential as a target for antifilarial drug development and for alternative therapeutic strategies toward onchocerciasis elimination

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SÃO TOMÉ AND PRÍNCIPE ON THE PATH TO ELIMINATING LYMPHATIC FILARIASIS

Jessca Vicente Fernandes

National center of endemic dissease, São Tomé, Sao Tome and Principe

Parasites — Helminths — Other Nematodes – Epidemiology and ControlLymphatic filariasis is a debilitating and disfiguring parasitic disease transmitted by infected mosquitoes. Usually acquired in childhood, it manifests in adulthood with conditions such as lymphoedema and hydrocele, leading to temporary or permanent disability. It affects over 120 million people in tropical and subtropical regions, including São Tomé and Príncipe, where it is endemic. Prior to the the interventions, 1,413 people were tested across the seven health districts, with a positivity rate of between 1% and 6% (FTS and thick smear). All districts were classified as endemic for LF. The first mass treatment campaign (MT-DA) was carried out in 2018, with 100% geographical coverage and 84.5% therapeutic coverage. In 2019, the country introduced triple therapy (IDA), achieving a therapeutic coverage of 75.7%, followed by a second round in December 2020, with coverage of 76.16%. Cross-sectional surveys were conducted, including individuals aged 20 years or older. The selection of locations and sample size was carried out using the Survey Sample Builder (SSB) tool, adapted for tritherapy impact assessments (IDA). Diagnosis was performed using the (FTS) rapid test for the detection of W. bancrofti antigen. All individuals with a positive FTS result underwent a night-time microfilariae examination using calibrated thick smears, with all results being negative. TAS 1 (2022): 14,132 people were surveyed in 153 localities. There were 21 FTS-positive cases, all of which were negative for microfilaremia. TAS 2 (2024–2025): 14,132 people were surveyed in 152 localities. Seven FTS-positive cases were recorded, all were also negative for microfilaremia. These findings confirm that the country is on track to eliminate LF as a public health problem with no resurgence of LF transmission four years after the last MDA.

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IDENTIFICATION OF ANTIBODY BIOMARKERS FOR *NECATOR AMERICANUS* AND *ASCARIS LUMBRICOIDES*

Yong Wang[1], Maurice Royal[2], **Sylvia Ossai**[1], Kimberly Y. Won[3], Sukwan Handali[1], W. Evan Secor[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Synergy, Atlanta, GA, United States, [3]Centers for Disease Control & Prevention, Atlanta, GA, United States

Soil-transmitted helminths (STHs) infect over 1 billion people globally, contributing to an estimated 2.2 million disability-adjusted life years, primarily due to impaired growth, cognitive development, and productivity loss. The current reference diagnostic method, stool microscopy, is highly specific but lack sensitivity in low-transmission settings. Antibody-based diagnostics offer promising utility for initial mapping, post-mass drug administration surveillance and integration into neglected tropical disease control programs. We performed *in-silico* analysis of *Ascaris lumbricoides* and *N. americanus* proteomes to identify candidate antigen for serological detection. A total of 78 *A. lumbricoides* and 51 *N. americanus* proteins were selected and expressed in a bacterial expression system. All *A. lumbricoides* proteins were successfully expressed, with 11 candidates demonstrating reactivity to *A. lumbricoides*-positive sera for IgG1 and/or IgG3 antibodies but not to uninfected individuals. For *N. americanus,* 48 proteins were successfully expressed, of which 31 showed reactivity with *N. americanus*-positive sera—predominantly IgG1—while showing no reactivity with sera from uninfected individuals. All reactive proteins will undergo further purification and be evaluated using a multiplex bead assay platform to assess diagnostic performance and cross-reactivity. This work aims to identify serological biomarkers capable of detecting ascariasis and hookworm infections and supporting integrated surveillance for STH control and elimination efforts.

6793

PRESENCE OF *ONCHOCERCA VOLVULUS* AND *MANSONELLA SPECIES* IN SKIN SNIPS AND ITS IMPACT ON ACCURATE DIAGNOSIS OF ONCHOCERCIASIS IN ENDEMIC AREAS.

Millicent Selassie Afatodzie[1], Yvonne Ashong[1], Millicent Opoku[1], Abena Akyeamaa Nyarko[1], Sarah-Sally Mawunyo Dogbe[1], Grace Opoku Gyamfi[1], Kwasi Agyenkwa-Mawuli[1], Emmanuel Frimpong Gyekye[1], Seth Offei Addo[1], Bethel Kwansa-Bentum[2], Fred Aboagye-Antwi[2], Dziedzom Komi de Souza[1]

[1]Noguchi Memorial Institute for Medical Research, Accra, Ghana, [2]University of Ghana, Accra, Ghana

Co-endemicity of *Onchocerca volvulus* and *Mansonella* spp poses challenges to diagnostic testing and the evaluation of Onchocerciasis elimination programmes. *Mansonella* spp, especially *M. streptocerca*, which can also be found in the skin, can be misdiagnosed as *O. volvulus*, potentially leading to false positives by microscopy. This study aimed to confirm the presence of *Mansonella* spp. among individuals in onchocerciasis-endemic areas in Eastern Ghana. Skin snip samples from 906 individuals were analyzed by microscopy for microfilaria detection, O-150 qPCR for the detection of *O. volvulus* and MP-R1 qPCR for the detection of *M. perstans*. 16 samples were positive by microscopy, 26 by O-150 qPCR and 6 by MP-R1 qPCR. Of the 16 microscopy-positive samples, 10 could not be confirmed by qPCR. Sanger sequencing and analysis of microscopy-positive and qPCR-positive samples yielded 14 sequences of sufficient quality for analysis. Three of the sequences had 83-95% similarity to *M. streptocerca*, and three had 78-90% similarity to *M. perstans*. 11 sequences were *O. volvulus* with 96-99% similarity to *O. volvulus* isolate. These results suggest the presence of *M. streptocerca* and *M. perstans* in the skin of community members in the study area, with implications for overestimating *O. volvulus* prevalence in endemic areas based on skin snip microscopy alone. Further studies are needed to estimate the prevalence of *Mansonella* spp. in these areas to inform public health decisions.

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POST-ELIMINATION ENTOMOLOGICAL SURVEILLANCE FOR ONCHOCERCIASIS IN THE FORMERLY ENDEMIC FOCI OF WESTERN KENYA, SEPTEMBER 2025

Sophia Moraa Ayienga[1], Mary Amuyunzu-Nyamongo[2], Wycliff P. Omondi[1], Milton Bwibo[3], Diana Kinagu[4], Nirote Mwirigi[1], Abdinasir Amin[5], Nancy Kinyua[6], John K. Kochey[7]

[1]Ministry of Health, NAIROBI, Kenya, [2]African Institute for Health and Development (AIHD), Nairobi, Kenya, [3]African Institute for Health and Development (AIHD), NAIROBI, Kenya, [4]Africa Institute for Health and Development (AIHD), NAIROBI, Kenya, [5]The END FUND, Nairobi, Kenya, [6]Independent Data and GIS Consultant, NAIROBI, Kenya, [7]National Museums of Kenya, NAIROBI, Kenya

Kenya is pursuing verification of onchocerciasis elimination following historical vector control using Dichlorodiphenyltrichloroethane (DDT) & vegetation clearing, and recent elimination mapping suggested transmission interruption in formerly endemic foci. To support national verification, post-elimination surveillance was conducted in formerly endemic & ecologically suitable counties in Western Kenya. A cross-sectional entomological survey & social assessments were conducted across 15 counties: Siaya, Homa Bay, Kisumu, Nandi, Kericho, Bomet, Kisii, Nyamira, Migori, Bungoma, Uasin Gishu, Trans Nzoia, Busia, Vihiga, & Kakamega. Selected rivers & streams were assessed for blackfly breeding. Larvae & pupae of *Simulium* spp. were collected from submerged rocks, trailing vegetation & other substrates. Freshwater crabs were trapped & examined for phoretic association with *S. neavei*. Structured community interviews captured blackfly biting history, and adult blackfly collection sites were established. Environmental water quality parameters were recorded too. Findings varied across counties. Strongest signals were observed in Bungoma (*S. neavei* larvae on crabs, though there were no reported bites) & Kakamega (multiple rivers with simulium larvae/pupae & reported bites). Busia, Homa Bay, Siaya, Nyamira & Nandi also showed vector evidence, including simulium larval/pupal positivity & localized biting history. Other counties showed focal or patchy larval/pupal positivity, Adult collections are ongoing at sites with both larval positivity & reported bites. In conclusion, widespread transmission was not observed, but some river systems still showed blackfly activity. Continued surveillance, species identification, lab testing & repeat seasonal surveys are needed to support post-elimination monitoring & Kenya's verification process.

6795

SYSTEMATIC MONITORING TO DETECT THE 'NEVER TREATED' POPULATION IN ONCHOCERCIASIS ELIMINATION PROGRAMS IN EDO SPECIAL INTERVENTION ZONE, SOUTHERN NIGERIA

Emmanuel Emukah[1], Emily Griswold[2], Hannah Lawinger[2], Lindsay Rakers[2], Jenna E. Coalson[2], Stephanie Palmer[2], Adamu Sallau[1], Bertram E.B. Nwoke[3], Emmanuel Miri[1], Solomon Adelamo[1], Izebhua B. Ikponmwosa[1], Lazarus Nweke[1], Egeonu Attamah-Isiani[1], Happy Poko[4], Chukwuemeka Makata[5], Fatai Oyediran[5], Gregory S. Noland[2]

[1]The Carter Center - Nigeria, Jos, Nigeria, [2]The Carter Center, Atlanta, GA, United States, [3]Imo State University, Owerri, Nigeria, [4]Edo State Primary Health Care Development Agency, Benin City, Nigeria, [5]Federal Ministry of Health, Abuja, Nigeria

Nigeria's national program began targeting onchocerciasis for elimination in 2013, calling for mass drug administration (MDA) to increase from annual to bi-annual in high-risk areas, such as the designated 'special intervention zone' (SIZ) on the Edo and Ondo state borders along the Osse River. The impact of biannual MDA may be limited if some people are never treated and can act as potential reservoirs harboring parasite infections. We evaluated the 'never-treated' population in recent MDA coverage surveys in the SIZ. Cluster sampling was used to systematically select communities and households in three districts of the Edo state SIZ: Akoko Edo, Esan West, and Etsako Central. The coverage survey was conducted in February 2026, following the November/December 2025 round of ivermectin MDA. All members of selected households were interviewed about their MDA uptake in the most recent round, all prior rounds, and reasons for treatment or non-treatment. We interviewed 3,892 persons, comprising 2,062 (53%) females and 115 (3%) children under 5 years. The surveyed coverage for the most recent round of MDA was 87.5% overall, ranging from 75.3% in Esan West to 93.4% in Akoko Edo. Excluding children under 5 who were not yet eligible for ivermectin MDA, 5.0% reported never taking MDA in this or prior rounds, ranging from 2.6% in Akoko Edo to 11.1% in Esan West. Among those 5 years and older there was no significant association between never-treatment and age (p=0.90) or gender (p=0.93); 5.2% of school-aged children 5-15 yrs, 5.0% of young adults 16-30 yrs, and 4.9% of older adults were never-treated and 5.0% of women and 4.9% of men were never-treated. Reasons given for never-treatment among those 5 and older were not knowing about MDA (24.9%), other ineligibility (22.7%), that drug distributors did not come (19.5%), absence during MDA (10.8%), and side effects (8.7%). Although the proportion of never-treated people in these study areas was small, particularly among those 5 years and older, we recommend systematic monitoring of never-treated populations to ensure that transmission can be interrupted with MDA.

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AGGRESSIVE BONE HYDATID CYST OF THE HIP: A RARE LESION WITHDIAGNOSTIC UNCERTAINTY OR DIFFICULTY IN MANAGEMENT

Kamena Mwana Yile Dady Dady Hassan[1], Bienvenu Jean Celien Okouango[2], Mustapha Fadili[3], Latifa MARIH[4]

[1]Infectious Diseases Department, Ibn Rochd University Hospital, Casablanca, Morocco, [2]Traumatology and Orthopaedics Department, Ibn Rochd University Hospital, HASSAN II University, Casablanca, Morocco, Casablanca, DC, United States, [3]Traumatology and Orthopaedics

Department, Ibn Rochd University Hospital, HASSAN II University, Casablanca, Morocco, Casablanca, WA, United States, [4]Infectious Diseases Department, Ibn Rochd University Hospital, Casablanca, DC, United States

Hydatid disease, or echinococcosis, is a zoonosis caused by the development in humans of the larval form of a tapeworm: Echinococcus granulosus. It is an anthropozoonosis that presents with various anatomical and radiological forms, with topographies associated with the progressive development of cysts. Bone hydatid disease, which is rare, accounts for less than 2.5% of cases. We report the case of a 57-year-old woman admitted for lameness and a mass in her left thigh, revealing a hydatid cyst of the iliac bone, hip joint, and femur. The lesion assessment revealed a hydatid cyst of the left hemipelvis involving the iliac bone, hip joint, femur, and adjacent soft tissues. Bone hydatid disease, an infiltrative, diffuse, slow, and progressive condition, leads to delayed diagnosis, compromising the quality of care and quality of life.

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INFECTION WITH LYMPHATIC FILARIASIS FIVE YEARS AFTER STOPPING MASS DRUG ADMINISTRATION IN LANGO REGION OF UGANDA

Gabriel Matwale[1], Stevens Kisaka[2], **Fred Ntoni Nuwaha**[2]

[1]Ministry of Health, Kampala, Uganda, [2]Makerere University, Kampala, Uganda

The World Health Organization launched the Global Programme to Eliminate Lymphatic Filariasis (LF) in 2000, which aimed at eliminating the disease by 2030. This goal mainly depends on mass drug administration (MDA). In Uganda MDA commenced in 2002 using Ivermectin and Albendazole. From 2019, all the 66 districts endemic for LF had stopped MDA. We measured the prevalence of infection with lymphatic filariasis in Lango, a previously endemic region (antigenemia of about 30% in 2001) five years after stopping of MDA. We also identified factors influencing infection with a view to sustaining the elimination gains. In February of 2021 1442 Individuals (from 30 randomly chosen clusters) who were five years and older were tested for *Wuchereria bancrofti* circulating filarial antigens using the Alere® Filariasis Test Strip (Abbot, Scarborough, ME). Addition data was collected from study participants such as age, sex, use of insect treated mosquito nets, use of indoor residual spraying and participation in previous MDA. The study was conducted in Lango of northern Uganda that lie between 1°30′ N and 2°44′ N and between 32°15′ E and 33°15′ E. Lango lies at an elevation of 900 to 1,200 meters. Lymphatic filariasis is predominantly transmitted by Anopheles mosquitoes. The overall prevalence of Lymphatic filariasis was 7/1442 (0.485% Confidence interval (CI) 0.235-0.999). LF was more common in males (5/629, 0.795%) compared to females (2/806, 0.248%). The prevalence was slightly lower among those who received MDA (5/764, 0.654%) compared to those who did not 1/95, 1.05%). Prevalence was higher among adults aged 18 years and above (6/825, 0.727%) compared to children (1/510, 0.196%); but lower among those who reported frequently using bed nets (3/978, 0.307%) versus those who never used bed nets (3/74, 4.05%). Furthermore, prevalence was comparable among those who reported indoor residual spraying (6/74, 0.810%) and those who did not (1/118, 0.847%). The study finding shows that lymphatic filariasis prevalence is low and below the threshold for MDA. This study indicated that those who were positive for LF were less likely to be using bed nets.

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CONTINUOUS HIGH CASES OF DIPHTHERIA IN INDONESIA: TWELVE-YEARS REPORT FROM EAST JAVA PROVINCE

Dominicus Husada[1], Rahma I. Mustikasari[2], Leny Kartina[3], Dwiyanti Puspitasari[1], Parwati S. Basuki[3], Ismoedijanto Moedjito[3], Zumaroh Zumaroh[4], Hugeng Susanto[4], Sulvy D. Anggraini[4], Erwin A. Triyono[4]

[1]Dr. Soetomo Hospital, Department of Child Health and Faculty of Medicine Universitas Airlangga, Surabaya, Indonesia, [2]Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia, [3]Dr. Soetomo Hospital, Department of Child Health, Surabaya, Indonesia, [4]East Java Provincial Health Office, Surabaya, Indonesia

Indonesia is currently ranked second in the world in diphtheria cases. East Java province has the most cases in this country. Despite many efforts to solve the problem, the high number of diphtheria cases has continued for at least twelve years. This study aims to explain the diphtheria situation in East Java Province since 2013. This cross-sectional surveillance report-based study used data from 38 districts in East Java from 1 January 2013 until 31 December 2024. Collected data included demographics, clinical information, additional examinations, immunization history, and close contact management. The reports were collected daily, weekly, and monthly at the provincial health office. The inclusion criteria for this report were: had a diagnosis of diphtheria and live in East Java province. The comparison was also made among data before 2020, during the pandemic era, and the 2023-2024 period. Based on the national guidelines, diphtheria-confirmed cases could be based on microbiological culture or a consensus decision from the National Diphtheria Expert Committee. There were 4221 cases during twelve years, and 53.9% were male. The biggest age group was 1-18 (2868=68.1%), especially between 60 and 145 months. The older adults and infant groups were only 1.2% and 1%, respectively. The majority of cases were tonsillopharyngeal diphtheria. The case fatality rate was 2.2% (94). Districts or cities with the most cases were Surabaya, Malang, Blitar, Sidoarjo, and Bangkalan. Cases from the west and south regions were predominant. Microbiological cultures were positive in only 13.39% of patients. Around 20% of cases had complete immunization by age and based on parental memory. Cases from pre, during, and after the pandemic era were 3408, 333, and 480 patients, respectively. In conclusion, the high number of diphtheria cases in East Java Province continues. Low immunization coverage may still be the main cause. Most patients were children and adolescents with incomplete or zero immunization status. The number of patients after the pandemic era was already similar to that before 2020.

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DIFFERENTIAL GENE EXPRESSION IN THE UPPER RESPIRATORY TRACT OF HUMANS INFECTED WITH SARS-COV-2 REVEALS VIRAL INFECTION BIOMARKERS

Andrea E. Luquette, Anthony Cicalo, Maren C. Fitzpatrick, Ghyssella E. Valdiviezo, J. Alexander Chitty, Gregory K. Rice, Regina Z. Cer, Cameron V. Sayer, Francisco Malagon, Kimberly A. Bishop-Lilly

Naval Medical Research Command, Frederick, MD, United States

SARS-CoV-2 is the causative agent of COVID-19, an infectious respiratory viral disease with human-to-human transmission. Current molecular understanding of how hosts respond to insults from respiratory viral pathogens in general and to SARS-CoV-2 in particular is still a research field under development. The activation levels of the different pathways are dependent on several variables, including host tissue compartment. In this work, we analyzed the transcriptional host responses to SARS-CoV-2 infections using nasopharyngeal (NP) swabs from infected and non-infected individuals. Differential gene expression (DGE) analysis identified 182 overexpressed genes, with strong enrichment in innate immune and viral response genes. Among them, we found a significant induction of IFIH1/MDA5, a pattern recognition receptor (PRR) gene participating in the initial sensing of viral RNAs and subsequent cascade activation of interferon (IFN) and IFN-stimulated genes (ISGs). Interestingly, RIG1 and TLR3, two other PRR genes that upregulate IFN and ISGs but which are not normally identified as differentially expressed genes (DEGs), have similar levels of induction. Moreover, OAS1, OAS2 and OAS3, three other PRR genes acting downstream in the activation cascade and promoting RNAseL-mediated apoptosis, are also among the upregulated genes. Finally, we confirmed by RT-qPCR the overexpression of MX1, a well characterized biomarker of viral infection; IFIT1, one of the top upregulated genes; and OAS1, OAS2 and OAS3, genes in chr12q24.13 COVID-19 susceptibility

locus. This study provides insights into upper respiratory tract responses to SARS-CoV-2 infections and identifies a set of DEGs with potential as viral infection biomarkers.

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INFLUENZA VIRUS AND SARS-COV-2 COCIRCULATIONS AND COINFECTIONS IN BURKINA FASO DURING THE POST-COVID-19 PANDEMIC ERA

Moussa Lingani[1], Kouadou H. Somé[2], Prisca Agathe Tarnagda[1], Wendlasida Marthe H. Kinda[2], Zekiba H. Tarnagda[2]

[1]Clinical Research Unit of Nanoro (CRUN), Siglé, Burkina Faso, [2]Institut de Recherche En Sciences de La Santé, Ouagadougou, Burkina Faso

In Burkina Faso, health authorities integrate COVID-19 surveillance with influenza to ensure continuous monitoring of both infections. The objective of this study was to evaluate the prevalence of concurrent influenza and COVID-19 infections in the post-pandemic period. This cross-sectional survey, conducted from January to August 2024 at four sites for influenza surveillance, included all patients exhibiting symptoms of acute respiratory infections. Patients' sociodemographic and medical data were collected, and pharyngeal swabs were tested by rRT-PCR for the detection of influenza virus and SARS-CoV-2. Of the 2,344 patients included, the prevalence was 8.8% (204/2306), 4.7% (108/2299), and 0.2% (5/2381) for influenza, COVID-19, and their coinfections, respectively. For influenza, subtype A(H3N2) was predominant (51.8%, 100/193), followed by B(Victoria) (23.3%, 45/193), A(H1N1)pdm09 (22.3%, 43/193), and type A coinfections (A(H1N1)pdm09+A(H3N2)) (2.6%). No case of B (Yamagata) was noted. By multivariate analysis, influenza prevalence significantly varied according to the patient's age group, the presence of a sore throat, and the severity of the infection ($p<0.05$). The patient's age group was, however, the only factor significantly associated with SARS-CoV-2 infections. The study also reported a low vaccination rate for both influenza and covid-19. The study reported in conclusion a persisting moderate cocirculation of influenza viruses and SARS-CoV-2, thus highlighting the risk of a resurgence. Reinforcing surveillance is needed to ensure adequate epidemic preparedness.

6801

PREVALENCE AND CLINICAL CHARACTERISTICS OF POST-TUBERCULOSIS LUNG DISEASE (PTLD) IN GAZA PROVINCE, MOZAMBIQUE

Troy Moon[1], Bartolomeu Chongo[2], Cecilia Wright[3], Elzier Mavume-Mangunyane[4], André Simango[2], Estevão Machava[2], Isabel Zacarias[2], Rogério Chiau[5], Gustavo Amorim[6], Kathryn T. Kampa[1], Harriett Meyers[1], Mohsin Sidat[4], Jerry Zifodya[7]

[1]Tulane University, Celia Scott Weatherhead School of Public Health and Tropical Medicine, New Orleans, LA, United States, [2]Carmelo Hospital of Chókwe, Chókwe, Mozambique, [3]Tulane University, New Orleans, LA, United States, [4]Universidade Eduardo Mondlane, Maputo, LA, United States, [5]The Aurum Institute, Mozambique, Maputo, Mozambique, [6]Vanderbilt University, Nashville, TN, United States, [7]Tulane University School of Public Medicine, New Orleans, LA, United States

Tuberculosis (TB) remains a leading cause of infectious disease-related mortalityworldwide. Despite successful treatment, many individuals experience persistent morbidity, mostnotably post-tuberculosis lung disease (PTLD). Mozambique is among the World HealthOrganization's 30 high burden countries for TB and TB/HIV co-infection. We aimed to determinethe prevalence and clinical characteristics of PTLD, including restrictive, obstructive, and mixedspirometric patterns. Repeated cross-sectional assessments for patients attending the National TBProgram clinic at Carmelo Hospital, Mozambique were taken, at TB treatment completion and sixmonths later. Eligible participants were ≥15 years old, had GeneXpert-confirmed TB, andsuccessfully completed 6-months of TB treatment. An ultrasound-based ndd EasyOne® spirometerwas used to define PTLD. Descriptive statistics summarized participant characteristics, and groupcomparisons were performed with chi-square and Mann–Whitney–Wilcoxon tests. Amongparticipants with acceptable spirometry, 54.7% had abnormal lung function consistent with PTLD.Compared with those without PTLD, affected participants had lower median weight and bodymass index (BMI), and significantly lower FEV1 and FVC z-scores. Overall respiratory symptomburden was low. Overall, self-rated health status using EQ-VAS was high, however, scores weresignificantly lower among those with PTLD compared to those without. In multivariable logisticregression, higher BMI was independently associated with reduced odds of PTLD. PTLDprevalence was high at six and twelve months after TB treatment initiation in Mozambique, in acontext of high national TB burden and no current in-country guidelines for how to manage post-TB treatment sequela. Low BMI was a potentially modifiable risk factor. Despite limited samplesize, these findings support routine screening for PTLD at the final TB clinic visit, as long-termlung function abnormalities are often already present. National treatment strategies which includeboth medication and pulmonary rehabilitation options are needed.

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EXAMINING BREASTFEEDING PATTERNS FOR THE EVALUATION OF ILLNESS SEVERITY IN CHILDREN AT RISK OF TUBERCULOSIS

Aida Doucoure[1], Domitila Augustino[2], Paulo Mejan[2], Joshua Mungi[2], Restituta Mosha[2], Mariamu Temu[2], Kelvin Msoka[2], Taylor Payne[1], Simeon Kusulla[2], Ghassan Ilaiwy[1], Tania Thomas[1], Esto Mduma[2], Saning'o Saruni Lukumay[2]

[1]University of Virginia, Charlottesville, VA, United States, [2]Haydom Lutheran Hospital, Haydom, United Republic of Tanzania

Breastfeeding is critical in early childhood nutrition, immune development, and protection against enteric infection. Particularly in tuberculosis (TB)-endemic settings, where undernutrition is increasingly recognized as the leading attributable risk factor for TB, affecting nearly 1 million children yearly. We aimed to evaluate the effect of breastfeeding status on hospital length of stay and TB-related discharge diagnoses among a cohort of hospitalized children <3 years of age. This is a substudy within a single-center prospective TB diagnostic study conducted in Northern Tanzania that enrolled children <10 years who were admitted with severe acute malnutrition (SAM) and/or pneumonia, and hospitalized controls; all were assessed for TB. Participants were <3 years of age and grouped by breastfeeding status. The primary outcome was hospital length of stay (LOS). T-tests were used to compare LOS by discharge diagnoses (TB, SAM, both, and hospitalized controls) and breastfeeding status. During the recruitment period of March 2024-2025, 158 children met eligibility criteria (62% male; mean age 1.15±1.84 years). TB was diagnosed in 4 (2.5%) participants, SAM in 53 (33.5%), concurrent TB+SAM in 11 (7%), pneumonia in (27.8%), and 46 (29.1%) participants were hospitalized controls. Twenty-eight (17.7%) children were exclusively breastfed (mean age 0.49±0.36 years), 51 (32.3%) were breastfed with complementary nutrition (mean age 0.88±0.42 years), and 79 (50%) were not currently breastfed (mean age 1.53±0.71 years). Children exclusively breastfed experienced longer hospital stays (mean=21.5±1.31 days) than those who were not exclusively breastfed (mean=18.8±8.7 days; $p=0.033$). Exclusively breastfed children with TB experienced longer hospital stays than those who were not (27±5.4 days vs.8±3.42 days, $p=0.004$). Additional multivariate regression adjusting for age, season of admission, and severity of illness are underway. Exclusive breastfeeding may serve as a sign of greater clinical vulnerability for children in TB-endemic settings, thereby warranting further exploration of contributing factors to this relationship.

6803

MEASLES VACCINATION INTENTIONS FOR CHILDREN AMONG CANADIAN ADULTS DURING NATIONAL OUTBREAKS

Hannah Melchinger[1], Amyn A. Malik[1], Krutika Kuppalli[2], Saad B. Omer[1], Jeanette Comeau[3], Ève Dubé[4], Noni MacDonald[4]

[1]Peter O'Donnell Jr. School of Public Health, UT Southwestern Medical Center, Dallas, TX, United States, [2]UT Southwestern, Dallas, TX, United

States, [3]Department of Pediatrics, Division of Infectious Diseases, Dalhousie University, Halifax, NS, Canada, [4]Department of Anthropology, Laval University, Quebec, QC, Canada

Since January 2025, Canada has reported over 5,998 cases of measles, resulting in the loss of its measles elimination status achieved in 1998. Over 90% of these cases have involved unvaccinated individuals or those with unknown vaccination status, and an estimated 20% have occurred in children under the age of five. Two infants have died. Vaccination remains the most effective protection against measles; however, measles attitudes and vaccination intention among Canadian adults are not well understood. In November 2025, we conducted a nationally representative survey to assess measles attitudes and vaccination intention among Canadian adults (18+). Participants were asked whether they would be willing to vaccinate a child under five against measles if recommended by a healthcare provider. Analyses were weighted using census estimations for sex, age, race, and education. Of 879 total respondents, 54% were female, 69% were over the age of 36, and 31% identified as a visible minority. Most (69%) had achieved at least a postsecondary education, and 40% reported household incomes over 80,000 CAD. 80% were willing to vaccinate a child under the age of five, and 12% were not. Among parents, 84% were willing to vaccinate a child against measles and 9% (were not; among non-parents, 76% were willing to vaccinate and 15% were not. Half of respondents (50%) reported an average knowledge of measles, 67% (63-70%) felt they could protect themselves from measles, and 47% (43-51%) felt the threat of measles was not being exaggerated. Population-level protection against measles requires >95% of the population be fully vaccinated. However, we found that only 80% of Canadian adults would be willing to vaccinate a child against measles. This finding highlights an important minority sufficient to breach the nominal threshold for herd immunity. Public health practitioners should seek to understand why this minority is unwilling to vaccinate their children and how to address those barriers. Effective interventions should target this minority with evidence-based interventions, including messaging and education.

6804

COVID-19 TRANSMISSION DYNAMICS AND TEMPORAL ASSOCIATIONS WITH NON-PHARMACEUTICAL INTERVENTIONS IN CALIFORNIA

Patra C. Ezeamii[1], Nenrot Gopep[1], Francis Barnabe Kengne[1], Jonathan Luyster[1], Jing X. Kersey[1], Xinyi Hua[2], Gerardo Chowell[3], Isaac Chun Hai Fung[1]

[1]Georgia Southern University, Statesboro, GA, United States, [2]The University of Kentucky, Lexington, KY, United States, [3]Georgia State University, Atlanta, GA, United States

California provides a large and heterogeneous setting to evaluate SARS-CoV-2 transmission dynamics and policy-associated changes in transmission potential. We examined how non-pharmaceutical interventions (NPIs) and urbanicity were temporarily associated with the time-varying reproduction number (Rt). Daily confirmed case count data from the Johns Hopkins University repository (January 2020-February 2023) were used to estimate Rt statewide and across six Public Health Regions. Infection dates were reconstructed via deconvolution. Infection counts were estimated with a multiplier of 4. Rt was estimated using a 7-day sliding window in EpiEstim. Four regions were further stratified into metropolitan and rural subregions. Policy impacts were quantified as percentage changes in Rt across non-overlapping time windows before and after five major NPIs implemented in 2020. Sensitivity analyses were conducted with alternative serial interval distributions and Rt prior distributions. Four epidemic waves were observed (winter 2020-2021; summer 2021; winter 2021-2022; mid-2022) at state-level. Specifically, Rt remained near the epidemic threshold ($Rt \approx 1$), with brief excursions above 1 preceding major surges. NPIs were temporarily associated with modest but consistent directional changes in transmission. The stay-at-home order corresponded to reductions in Rt of 2-6%, and face mask mandates to reductions of 3-8%, with the strongest effects in the Bay Area and Greater Sierra-Sacramento region. Stage 2 reopening was associated with increases of 1-6%, and extension of roadside business operations with increases of 4-10%, particularly in Los Angeles and the Bay Area. Stratified analyses suggested larger magnitude associations in metropolitan areas, whereas rural areas showed reduced and less precise estimates. NPIs were temporarily associated with modest changes in transmission, with patterns indicating heterogenous and limited shifts in epidemic dynamics. The magnitude of estimated changes varied by urbanicity, emphasizing the importance of geographically tailored public health strategies in future outbreaks.

6805

HOUSEHOLD AIR POLLUTION IS ASSOCIATED WITH DISEASE SEVERITY AND MORTALITY IN UGANDAN CHILDREN HOSPITALIZED WITH HYPOXEMIC PNEUMONIA

John Kanyonyozi[1], Ayla Ahmed[2], Sehaj Sandhu[3], Sophie Namasopo[4], Juliet Nabwire[1], Qaasim Mian[3], Andrea L. Conroy[5], Jackson Amone[6], Charles Olaro[1], Robert Opika Opoka[7], Michael T. Hawkes[8]

[1]Association for Health Innovation in Africa, Kambuga, Uganda, [2]University of St. Andrews, St. Andrews, United Kingdom, [3]University of Alberta, Edmonton, AB, Canada, [4]Hoima Regional Referral Hospital, Hoima, Uganda, [5]Indiana University, Indianapolis, IN, United States, [6]Ministry of Health, Kampala, Uganda, [7]Aga Khan University, Nairobi, Kenya, [8]University of British Columbia, Vancouver, BC, Canada

Household air pollution (HAP) due to biomass fuel use is a risk factor for childhood pneumonia, a leading cause of under-five mortality globally. This study examined the relationship between HAP and disease severity in Ugandan children hospitalized with hypoxemic pneumonia. We conducted a retrospective case-control study across 20 Ugandan hospitals. $PM_{2.5}$ exposure was estimated using caregiver-reported fuel use, cooking duration, kitchen structure, and ventilation. Pneumonia severity was assessed clinically, and associations were analyzed using non-parametric tests. We included 735 children (median age 9 months, 42% female) hospitalized with hypoxemic pneumonia. Most households used firewood (84%) or charcoal (16%) for cooking. Other HAP sources included cigarette smoke (17%) and open-flame lighting (17%). The median estimated personal $PM_{2.5}$ exposure was 145 µg/m³ (IQR 79-270), and 732 children (99.6%) exceeded the recommended WHO limit of 15 µg/m³. Chronic HAP-related symptoms included cough (57%), red eyes (41%), rhinorrhea (38%), difficulty breathing (22%), and wheeze (6.5%). Higher PM2.5 exposure was significantly associated with more frequent red eyes ($p=0.0073$), rhinorrhea ($p=0.016$), and difficulty breathing ($p<0.0001$). The median SICK score was 3.9 (IQR 3.4-5.0). Higher scores correlated with higher $PM_{2.5}$ exposure ($\tau=0.15$, $p<0.0001$) and increased mortality risk ($p=0.0024$). Higher $PM_{2.5}$ exposure was also linked to WHO danger signs, lower SpO_2 at admission, longer duration of oxygen therapy, and greater total oxygen volume administered over the hospital admission ($p<0.05$ for all). Household $PM_{2.5}$ exposure from biomass combustion is associated with greater pneumonia severity in hospitalized children under five. Reducing household air pollution through cleaner fuels, better ventilation, and behavioral changes may improve outcomes for a leading cause of child mortality globally.

6806

CAREGIVERS' RISK PERCEPTION AND IMMUNIZATION STATUS OF UNDER-FIVE CHILDREN AGAINST DIPHTHERIA IN RURAL COMMUNITIES OF OYO STATE, NIGERIA: A MIXED METHOD STUDY

Mobolaji Modinat Salawu, Magbagbeola David Dairo, Olufunmilayo Ibitola Fawole

College of Medicine, University of Ibadan, Ibadan, Nigeria

Diphtheria, a highly contagious vaccine-preventable disease has re-emerged in Nigeria. Rising incidence of the infection is reported especially among children, with about 10% mortality. This study assessed the knowledge, risk perception of caregivers and immunization status of under-five children towards Diphtheria in rural communities of Oyo State, Nigeria. A cross-sectional study was conducted in 2025 among 566 caregivers selected by multistage sampling technique from rural communities. Mixed method of data collection utilized pretested semi-structured questionnaire,

Focus Group Discussion (FGD, n=56) and child immunization cards. Knowledge and perception of caregivers were assessed and categorised into poor and good/high respectively. Associations were examined using binary logistics regression at 95% confidence intervals (CI). Interviews were transcribed and analysed using Nvivo. Mean age of the caregivers and under-five children were 50.7±7.5 years and 22.4±15.9 months, respectively. Thirty-one percent had primary education and below, and 87.4% were ever married. Only 25.8% of the caregivers had ever heard of Diphtheria and 13% knew it could be transmitted from infected person through coughing and sneezing. About 26.9% reported Diphtheria is a dangerous infection and any child can be at risk (16.4%). Overall, 12.6% had good knowledge, and 76.1% had high risk perception towards Diphtheria infection. About 65.9% of the children had received Diphtheria vaccine, mostly from government facilities (64.7%). Caregivers who were ever married (adjusted odds ratio (aOR)=3.5, 95%CI:1.1-11.5) had higher likelihood of having good knowledge, and high-risk perception (aOR=3.2, 95%CI:1.4-7.3) towards Diphtheria. FGD participants also reported poor knowledge of Diphtheria. Some participants were hesitant while others expressed confidence about Diphtheria vaccination. Knowledge was poor among the caregivers, but risk perception towards Diphtheria was high. Immunization status of the children was also suboptimal. Awareness, education of caregivers about Diphtheria and improved vaccine uptake of children is crucial.

6807

GEOGRAPHIC VARIATION IN ANTIBODY RESPONSES AND SELECTION ON *MYCOBACTERIUM TUBERCULOSIS* ANTIGENS CHALLENGE THE DEVELOPMENT OF TB DIAGNOSTICS

Mei-Hsuan Wu[1], Dai-Wei Huang[1], Pin-Xuan Chen[1], Ying-An Angie Chen[2], Xiaowu Liang[3], Brian Arnold[4], Andy Teng[3], Jozelyn V. Pablo[3], Angela Yee[3], Cheng-Sheng Lee[1], Hsiao-Han Chang[1]

[1]National Tsing-Hua University, Hsinchu, Taiwan, [2]National Cheng Kung University, Tainan, Taiwan, [3]Antigen Discovery Inc, Irvine, CA, United States, [4]Stanford University, Stanford, CA, United States

Tuberculosis (TB) remains a major global infectious disease, and accurately distinguishing between latent TB infection (LTBI) and active TB (ATB) is critical for effective disease control. However, current diagnostic methods struggle to reliably differentiate these two states. Here, we evaluated the potential of protein microarrays to distinguish TB disease states and assessed how antigen evolution may influence their performance. We analyzed 426 serum samples from Vietnam, Bangladesh, Peru, and South Africa from the FIND biobank (www.finddx.org), measuring antibody responses against 3,963 *Mycobacterium tuberculosis* (*Mtb*) antigens. LTBI samples from Vietnam exhibited significantly higher antibody response intensities compared with ATB and non-TB (NTB), whereas minimal differences were observed between ATB and NTB. A LASSO model achieved 79.5% accuracy in distinguishing LTBI from ATB within Vietnamese samples, but performance declined substantially across geographic settings, indicating strong geographic specificity in antibody response profiles. Genetic diversity was not associated with variability in antibody responses, suggesting that other factors drive these geographic differences. Antibody response magnitude in LTBI was positively associated with the number of codons under positive selection, consistent with stronger selective pressure on these antigens. Overall, these findings highlight pronounced geographic specificity in antibody responses and evidence of selection acting on *Mtb* antigens, underscoring the importance of incorporating geographic and evolutionary context in the development of TB diagnostic tools.

6808

COMPARATIVE DNA EXTRACTION METHODS USED FOR LAMP ASSAY AS A MOLECULAR DIAGNOSIS OF HUMAN SCHISTOSOMES FROM URINE SAMPLES

Brittany Pulkilla[1], Chummy Sikasunge[2], James C.L. Mwansa[3], **Nilanjan Lodh**[1]

[1]Department of Medical Laboratory Science, Marquette University, Milwaukee, WI, United States, [2]Department of Para-clinical Studies, University of Zambia, Lusaka, Zambia, [3]University Teaching Hospital, Lusaka Apex Medical University, Lusaka, Zambia

Schistosomiasis in Africa is a persistent public health issue caused by two major human species, *Schistosoma mansoni* and *S. haematobium*, which often lead to concurrent infections. Due to the global goal of controlling or eliminating schistosomiasis as a public health problem, the issue of diagnostic sensitivity has become more critical in the assessment of program success. In that regard, the World Health Organization (WHO) has drawn attention to the need for field-applicable tests with high specificity and sensitivity. To address this, we have evaluated the amplification of *S. mansoni* and *S. haematobium* cell-free repeat DNA by loop-mediated isothermal amplification (LAMP) from field-collected filtered urine samples from 30 school children in Zambia. We have used four DNA extraction techniques (Qiagen and LAMP-PURE (LP): column-based DNA extraction technique, Chelex, and heating: rapid DNA extraction technique) to determine their impact on LAMP sensitivity and specificity, along with cost analysis and person-time involvement for each approach. Both Qiagen and LP extraction detected positive infections, but Qiagen extraction is more cost-effective than LP. DNA extraction by LP is the fastest (average 20 min.) compared to the other three methods, although it is the most expensive, including amplification ($9.35 compared to $4.90 for heating extraction and amplification). Chelex extraction is slower and simpler than LP and detects 20% more positive infections than heating. Heating extraction is very fast, inexpensive, and simple to perform. However, LAMP amplification for heating-extracted samples resulted in false negatives, possibly indicating the presence of inhibitor(s). In conclusion, We have demonstrated the sensitivity, cost-effectiveness, and time requirement of LAMP with four different DNA extraction approaches for the detection of two schistosome parasite species from a single field-collected urine sample.

6809

EXTENDING TREATMENT BY PRAZIQUANTEL TO ADULT POPULATIONS TO ENHANCE SCHISTOSOMIASIS CONTROL IN MADAGASCAR: PRELIMINARY FINDINGS

Alpha Alpha[1], Seheno Razanatsiorimalala[2], Dina Ny Aina Liantsoa Randriamiarinjatovo[2], Viviane Razafindravao[3], Arsène Indriambelo[1], Milijaona Randrianarivelojosia[2]

[1]Ecole Doctorale Géosciences, Physique et Chimie de l'Environnement et Système Hôtes- Pathogènes, Université de Toliara, Madagascar, Toliara, Madagascar, [2]Institut Pasteur de Madagascar, Antananarivo, Madagascar, [3]Clinique Médical Betela Andaboly, Toliara ; Madagascar, Toliara, Madagascar

In western Madagascar, urinary schistosomiasis caused by *Schistosoma haematobium* infection remains highly prevalent despite annual preventive chemotherapy with praziquantel targeting school-aged children since 2017. Transmission persists particularly in populations living near water bodies and irrigated rice fields. We conducted a community-based study in Amboboka and Andranokova, in a hot subarid area of southwestern Madagascar, to assess the impact of extending treatment by praziquantel to adults (≥15 years). In November 2024, urine samples were collected from randomly selected consenting adults. *S. haematobium* eggs were detected using the filtration method. Infected individuals received a single oral dose of praziquantel (40 mg/kg), delivered via community health workers. Follow-up was performed after three months. A total of 151 adults were enrolled (median age: 29 years, sex ratio: 1.05). Baseline prevalence of urinary schistosomiasis was 50.3% (95% CI: 42.1–58.5; 76/151), with a mean egg density of 207 ± 453 eggs/10 mL of urine (range: 1–3408). No household owned a latrine, and open defecation was common. Swimming

in local water bodies was identified as a major risk factor for infection. At follow-up, 130 participants were reassessed; and urinary schistosomiasis prevalence decreased to 21.5% (95% CI: 15.0–29.8; 28/130; $p < 0.05$); and a reduction in mean density to 51 eggs/mL of urine was recorded. Among positives, 4 were new infections. Twelve participants reported not taking treatment despite receiving it, citing poor palatability, fear of side effects, and redistribution within households. Adults represent an important reservoir for schistosomiasis transmission in this setting. Including adults in mass drug administration, alongside directly observed treatment and improved community engagement, may strengthen schistosomiasis control efforts in Madagascar. To provide proof of concept for expanded treatment strategies, repeated mass drug administration will be carried out for all residents aged ≥5 years in Amboboka and Andranokova.

6810

SCHISTOSOMIASIS CONTROL FROM A ONE HEALTH PERSPECTIVE (SCOPE) STUDY PHASE II: LONGITUDINAL IMPLEMENTATION OF AN INTERDISCIPLINARY SCREENING INTERVENTION IN RURAL NIGERIA

Simon Birk[1], Caroline Crain[1], William C. McCarthy[1], Ifeoluwa George[2], Soneye Islamiat[3], Cynthia Umannakwe[4], Tope Olubodun[5], Makinde Adebayo Adeniyi[5], Adedayo Oludolamu[5], Cristina Salas[6], Rakesh Kathiresan[1], Madison Horn[1], Megan Ngai[1], Manu Prakash[7]

[1]UTHealth McGovern Medical School, Houston, TX, United States, [2]Federal University of Agriculture Abeokuta, Abeokuta, Nigeria, [3]Neglected Tropical Diseases Unit, Department of Public Health, Ogun State Ministry of Health, Abeokuta, Nigeria, [4]Federal University of Medicine and Medical Sciences, Abeokuta, Nigeria, [5]Federal Medical Center, Abeokuta, Nigeria, [6]John Sealy School of Medicine at UT Medical Branch, Galveston, TX, United States, [7]Stanford University, Stanford, CA, United States

Schistosomiasis is a neglected tropical disease that is broadly endemic across sub-Saharan Africa, where prevalence exceeds 50% in many hyper-endemic communities. The urogenital form of the disease is a major cause of preventable morbidity, linked to hematuria, bladder cancer, and infertility. Mass drug administration of praziquantel to children is the primary control strategy but is resource-intensive and faces serious implementation barriers in last mile settings. However, alternative approaches rely on diagnostics that are traditionally centralized and laboratory dependent, posing similarly challenging logistical and structural constraints. This study will consist of a three-cluster, stepped wedge implementation study involving 18 rural communities in Ogun State, Nigeria over a period of 11 months. Community health extension workers will be trained on a novel, low-cost, and point-of-care diagnostic toolkit consisting of the Foldscope, a commercially-available, field-friendly microscope, and a reusable urine filtration device designed by this team. Three implementation strategies, including parallel educational initiatives, will be trialed by cluster. Outcome measures include accuracy of the toolkit, changes in pre- and post-intervention community-level prevalence, and qualitative measures of feasibility. This abstract will present preliminary data collected during the 2026 study period. Our proposal enables a shift from episodic mass treatment to continuous, community-led identification, treatment, and prevention of infection. With an emphasis on feasibility and diagnostic task-shifting, this work lays the foundation for community-led control programming that can scale across Nigeria.

6811

IMPLEMENTATION OF A REAL-TIME QPCR ASSAY FOR THE DIAGNOSIS OF FASCIOLA HEPATICA IN STOOL SAMPLES FROM ENDEMIC AREAS IN CUSCO, PERU.

Martha V Fernández-Baca[1], Ramos-Lette Diego M[2], Maria L Morales[3], Melinda B. Tanabe[4], Pedro Ortiz[5], A. Clinton White Jr[4], **Alejandro Castellanos-Gonzalez**[4], Miguel M Cabada[4]

[1]Universidad Peruana Cayetano Heredia-University of Texas Medical Branch Collaborative Research Center-Cusco, Cusco, Peru, [2]Universidad Peruana Cayetano Heredia-University of Texas Medical Branch Collaborative Research Center-Cusco, Galveston, Peru, [3]Universidad Peruana Cayetano Heredia, Cusco, Peru, [4]University of Texas Medical Branch, Galveston, TX, United States, [5]Universidad Nacional de Cajamarca, Cajamarca, Peru

Human fascioliasis remains a significant global health burden, particularly in the Andean highlands of Peru where the Cusco region exhibits some of the highest reported prevalence rates in the world. Despite the critical need for effective surveillance in these endemic communities, clinical validation of qPCR assays using human stool samples remains scant. Because traditional microscopy is hindered by intermittent egg shedding, this study implemented a more stringent reference standard of two independent Kato-Katz examinations to evaluate qPCR performance in 307 clinical samples from Cusco, Peru. Following collection, stool samples were stored frozen to maintain nucleic acid integrity prior to processing. DNA was extracted using the E.Z.N.A.® Stool DNA Kit and subsequently quantified by NanoDrop spectrophotometry to ensure adequate template quality. The qPCR assay demonstrated a sensitivity of 81.0% and a specificity of 91.7%, with Kappa analysis indicating substantial agreement and McNemar's test showing no significant difference between the methods. These results underscore the importance of using multi-point microscopic references for diagnostic validation and confirm that qPCR is a robust, highly specific tool for clinical detection in high-altitude endemic zones. By addressing the current gap in field-based molecular validation, this study supports the integration of qPCR into One Health surveillance strategies to improve early case detection and mitigate the impact of this neglected zoonotic disease.

6812

DEVELOPMENT OF A RAPID MINIPCR LATERAL FLOW ASSAY (LFA) FOR THE DIAGNOSIS OF *FASCIOLA HEPATICA* IN STOOL SAMPLES FROM ENDEMIC AREAS IN CUSCO, PERU

Alejandro Castellanos-Gonzalez[1], Ramos-Lette Diego M[2], Martha V Fernández-Baca[3], Melinda Tanabe[1], Maria Luisa Morales[4], Pedro Ortiz[5], A. Clinton White Jr[1], Miguel Mauricio Cabada[1]

[1]University of Texas Medical Branch, Galveston, TX, United States, [2]Universidad Peruana Cayetano Heredia-University of Texas Medical Branch Collaborative Research Center-Cusco, Cusco, Peru, [3]Universidad Peruana Cayetano Heredia-University of Texas Medical Branch Collaborative Research Center-Cusco, Galveston, Peru, [4]Universidad Peruana Cayetano Heredia, Cusco, Peru, [5]Universidad Nacional de Cajamarca, Cajamarca, Peru

Fascioliasis is a major zoonotic burden in the Peruvian highlands, where human prevalence can exceed 40%. Effective "One Health" surveillance is currently hampered by the low sensitivity of traditional microscopy, which fails to detect early-stage infections. While qPCR provides a highly sensitive molecular alternative, its reliance on expensive RT-PCR thermocyclers makes it unfeasible for low-resource, endemic settings. To bridge this gap, combining portable miniPCR technology with lateral flow assays (LFA) offers a field-deployable alternative. Our goal was to develop and validate a rapid miniPCR-LFA diagnostic tool for the detection of *Fasciola hepatica* in stool samples from endemic areas in Cusco, Peru. F. hepatica DNA amplification was performed using a portable miniPCR thermocycler. For the LFA readout, we designed F. hepatica-specific primers dual-tagged with Biotin and FAM. The assay was validated using DNA purified from 67 human stool samples previously characterized by microscopy and laboratory-based qPCR in triplicate to establish a comparative gold standard. The miniPCR-LFA demonstrated a sensitivity of 92.52% and a specificity of 92.50% compared to qPCR. These results indicate that this portable molecular platform provides a highly accurate, field-ready alternative to conventional laboratory methods, facilitating early diagnosis and improved disease surveillance in resource-limited settings.

6813

SYSTEMATIC REVIEW OF SCHISTOSOMIASIS SEQUELAE: 1980-2025

Rachael E. Bokota[1], Cathleen Keller[2], Jason Q. Nguyen[1], Ewerton Cousin[1], Thejasshri Vembakkam[1], Matthew T. Schneider[1], Jonathan F. Mosser[1]

[1]Institute for Health Metrics and Evaluation, Seattle, WA, United States, [2]Institute for Health Metrics and Evaluation, Chicago, IL, United States

Schistosomiasis is a neglected tropical disease caused by multiple species of the genus Schistosoma, transmitted via contact with contaminated fresh water. There were approximately 186 million prevalent cases in 2023, primarily in low- and middle- income settings, constituting a substantial health burden. Despite its widespread impact, the full extent of morbidity is likely underrepresented in current estimates. We aim to evaluate the current landscape of data to quantify the excess prevalence of schistosomiasis sequelae. We systematically mapped potential sequelae via an initial scoping review and expert consultation. Due to the systemic nature of schistosomiasis, we conducted systematic reviews by organ system. We searched PubMed using specific search terms related to each associated sequela for studies published from 1980 to 2025. If fewer than 50 studies were found, Embase or Web of Science were also searched. We included studies from species-specific endemic locations, sample sizes of at least 50, and geographically representative populations. Studies were excluded if they used diagnostic methods inconsistent with current Global Burden of Disease Study methods. All results presented are preliminary. We identified 4,886 studies, with 190 selected after full-text screening. Included studies pertained to 52 sequelae identified in the initial scoping review and were classified into 10 groups. Data availability by sequelae group varied widely, with gastrointestinal pathology having the most studies, and cardiopulmonary pathology the least. The wide range of data availability by sequela signals a need for more research on the morbidity of schistosomiasis. This systematic review represents a quantitative analysis of the excess prevalence of schistosomiasis sequela to better estimate its burden.

6814

MICROHABITAT LEVEL RISK MODELING IDENTIFIES PERSISTENT SCHISTOSOMIASIS FOCI IN LOW-ENDEMIC DISTRICTS OF ETHIOPIA

Asrat Meleko[1], Naomi Caplan[1], Daniel Sher[2], Michal Bruck[1], Nisan Kesete[1], Bisrat Molla[1], Azeb Seifu[1], Willemijn Zaadnoordijk[3], Zvi Bentwich[1], Dorin Brener Turgeman[1], Noa Dahan[1]

[1]NALA, Tel Aviv-Yafo, Israel, [2]University of Haifa, Haifa, Israel, [3]Ares Trading S.A, Eysins, Switzerland

Schistosomiasis transmission is highly focal, persisting in discrete freshwater microhabitats that support intermediate host snails, particularly in low-endemic settings where transmission continues despite repeated mass drug administration. Identifying these residual hotspots is critical for targeted control. We conducted a cross-sectional malacological survey of 172 freshwater sites across eight districts in three regions of Ethiopia. Snail presence, abundance, and infection status were assessed alongside environmental characteristics and site-level human infection prevalence. To identify ecological drivers and enable risk stratification, we analyzed the collected data using statistical and machine learning approaches. Logistic regression identified predictors of snail presence, while generalized linear models assessed abundance. A random forest model integrated these variables to generate site-specific risk scores, with performance evaluated using 5-fold cross-validated AUC. Host snails were detected at 52.3% of sites, with significant spatial heterogeneity ($p<0.001$). Snail presence was strongly associated with muddy substrate (OR 22.3), dense vegetation (OR 22.0), and higher water temperature (OR 1.98). The model demonstrated high predictive performance (AUC=0.91±0.05) and classified 75 sites as high-risk, 37 as medium-risk, and 60 as low-risk, with high-risk sites showing higher human infection prevalence and infected snail counts ($p<0.01$). These findings demonstrate that microhabitat factors drive focal transmission of schistosomiasis and provide strong evidence supporting targeted interventions to accelerate elimination.

6815

PREVALENCE, INTENSITY, AND RISK FACTORS OF PERSISTENT UROGENITAL SCHISTOSOMIASIS IN HARD-TO-REACH COMMUNITIES IN THE MIDDLE BELT OF GHANA

Anabel Acheampong[1], Paulina Oduro[1], Prince-Charles Kudzordzi[2], Prince Nyarko[1], Dennis Ofori Kyei[1], Lydia Datsa[1], Conor R. Caffrey[3], Constance Schultsz[4], Henk DFH Schallig[4], Kenneth Otabil Bentum[1]

[1]University of Energy and Natural Resources, Sunyani, Ghana, [2]Consortium for Neglected Tropical Diseases and One Health, University of Energy and Natural Resource, Sunyani, BA, Ghana, [3]University of California San Diego, San Diego, CA, United States, [4]University of Amsterdam, Amsterdam, Netherlands

Ghana has made significant progress in the control of schistosomiasis since 2010, but the disease persists in some communities. The prevalence, intensity and risk factors of persistent urogenital schistosomiasis transmission in four hard-to-reach communities in the middle belt of Ghana were investigated to better understand this persistence. A cross-sectional study was conducted in the at-risk communities of Tato Battor, Kwaese, Gobu and Kpatsa in the Sene West District of the Bono East Region of Ghana. Urine samples were collected from 230 school-aged children for microscopic examination of *S. haematobium* eggs and dipstick chemistry to detect micro-haematuria. Questionnaires were also completed by participants to assess the risk factors associated with urogenital schistosomiasis. The 230 school-aged children had a median age of 10 (Interquartile range = 7-12 years) with 53.9% (124) of the study participants being male. The overall prevalence of micro-haematuria was 50.0% (115/230, 95% CI: 43.6% - 56.4%) and the overall prevalence of *S. haematobium* eggs was 50.9% (117/230, CI: 44.9% - 57.3%). The Gobu community had the highest prevalence of haematuria and the presence of eggs at 85.6 and 68.1%, respectively, whereas the lowest prevalence was found in Kpatsa with 17.5 and 40.0%, respectively. Residing in Gobu was associated with significantly greater odds of being infected with *S. haematobium*. However, for all communities, there was no association between socio-demography, risk factors and the prevalence of infection. The study revealed an unexpected high prevalence of haematuria and *S. haematobium* eggs in urine of school-aged children, indicative of substantial ongoing transmission of urogenital schistosomiasis. Our findings highlight the need for targeted action by the Ghana Neglected Tropical Diseases Programme, as well as better education regarding the transmission of this disease, if it is to be eliminated as a public health burden in the district.

6816

RISK FACTORS FOR CHRONIC FASCIOLIASIS IN RURAL COMMUNITIES OF THE NORTHERN AND SOUTHERN HIGHLANDS OF PERU

Melinda B. Tanabe (Wright)[1], Maria Luisa Morales[2], Felix Meza Chavez[2], Ebeling Rios[2], Jose L. Alccacontor[2], Cristian Hovan[3], Cesar A. Murga-Moreno[3], Carol Castro[2], Dayana M. Terrones-Cerna[2], Maricarmen Sequeiros[2], Ana M. Fernandez-Sanchez[2], Patricia Mallma Salazar[4], Cesar Carcamo Cavagnaro[4], Pedro Ortiz[3], Miguel Mauricio Cabada[5]

[1]University of Texas Medical Branch, Galveston, TX, United States, [2]Instituto de Medicina Tropical Alexander von Humboldt - Universidad Peruana Cayetano Heredia, Cusco, Peru, [3]Universidad Nacional de Cajamarca, Cajamarca, Peru, [4]Universidad Peruana Cayetano Heredia, Lima, Peru, [5]University of Texas Medical Branch, Dickinson, TX, United States

Human fascioliasis, caused by Fasciola hepatica, remains highly endemic in the highlands of Peru. The region is recognized as one of the highest global burdens of disease, underscoring the need to identify context-specific risk factors to inform targeted control strategies. We conducted

a cross-sectional study in rural communities of the northern (Cajamarca) and southern (Cusco) highlands, defining chronic infection by at least one positive stool sample using Kato-Katz and rapid sedimentation techniques, alongside structured surveys assessing sociodemographic characteristics, eating habits, water sources, agricultural practices, and livestock exposures. A total of 835 households comprising 2,114 individuals were included (52.8% from Cusco and 47.2% from Cajamarca), with a median age of 17 years (IQR 10-45), 57.6% female, and a median residence time of 14 years (IQR 9-40). Overall prevalence was 6.5%, higher in Cajamarca than Cusco (8.2% vs 4.9%). In multivariable analyses, having a higher probability of living below the international extreme poverty line (PR 1.71, 95% CI 1.35-2.15, p<0.001), drinking from irrigation canals (PR 2.58, 95% CI 1.51-4.41, p<0.001), and combined use of canals and artificial reservoirs (PR 1.98, 95% CI 1.25-3.15, p=0.004) were independently associated with higher infection prevalence, whereas age >18 years (PR 0.20, 95% CI 0.09-0.45, p<0.001) and consumption of raw tubercles (PR 0.51, 95% CI 0.35-0.73, p<0.001) were associated with lower prevalence of infection. No specific plant-based food exposures were linked to increased risk. These findings, consistent with prior regional literature, highlight untreated water sources as key drivers of transmission in endemic highland settings. Strengthening control measures—including improved access to safe water, health education, and integrated One Health approaches targeting livestock reservoirs and environmental contamination—is essential to reduce transmission and address the substantial, yet underrecognized, burden of human fascioliasis.

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HOUSEHOLD CLUSTERING OF *FASCIOLA HEPATICA* INFECTION IN THE COMMUNITY OF POLLOC, CAJAMARCA, PERU

César A. Murga-Moreno[1], David Ruiz-Perez[1], Cristian Hoban[1], Dayana M. Terrones-Cerna[1], Ana M. Fernández-Sánchez[1], Jhover Díaz-Muñoz[1], Sandra Quispe-Chuquiruna[1], Fabiano Cruzado-Chávez[1], José A. Aliaga-Tambo[1], Angie Chávez-Pérez[1], Alejandra Hoyos-Sangay[1], **Pedro Ortiz**[1], Miguel M. Cabada[2]

[1]Centro de Investigación en Medicina Tropical: Fascioliasis, Facultad de Ciencias Veterinarias, Universidad Nacional de Cajamarca, Cajamarca, Peru, [2]Infectious Diseases Division, Internal Medicine Department, University of Texas Medical Branch, Galveston, TX, United States

Fasciola hepatica is highly prevalent in the Peruvian Andes. The risk of infection is higher among school-aged children and households. We conducted a cross-sectional study in the community of Polloc in Cajamarca, Peru, evaluating children with and without fascioliasis and their adult household members to determine the risk of fascioliasis among household members of children with the infection. Infections were diagnosed by microscopic testing of multiple stool samples using rapid sedimentation and Kato-Katz. Demographic, epidemiological, and socioeconomic information was collected. Associations with a positive test result in children were analyzed, crude prevalence ratios (PR) were calculated, and finally, a Poisson regression was performed to estimate the adjusted prevalence ratios (aPR). A total of 248 children and 168 adults from 133 households were tested. We identified 66 (26.7%, IC95% 21.5%-32.4%) positive children and 23 (13.7%, IC95% 9.3%-19.7%) positive adults. In the adjusted model, consumption of water from irrigation canals in the fields (aPR 1.87, 95% CI: 1.24-2.84; p = 0.003) and the presence of at least one adult testing positive for Fasciola in the household (aPR 1.74, 95% CI: 1.11-2.72; p = 0.016) were independently associated with F. hepatica positivity in children. No associations were identified for raw vegetable consumption, the source of vegetable acquisition, the household's drinking water source, or the ownership of sheep, cattle, or pigs. These findings suggest an association between fascioliasis cases within households and highlight the role of shared environmental exposures and cohabitation in maintaining the infection.

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SCHISTOSOMIASIS AND RELATED TREMATODE INFECTIONS IN HUMANS, LIVESTOCK, AND SNAILS IN NORTHWESTERN TANZANIA

Teckla Angelo[1], David J. Civitello[2], Paul Mireji[3], Safari Kinung'hi[4]

[1]Nelson Mandela African Institution of Science and Technology, Arusha, United Republic of Tanzania, [2]Emory University, Atlanta, GA, United States, [3]Kenya Agricultural and Livestock Research Organization, Kikuyu, Kenya, [4]National Institute for Medical Research, Mwanza Centre, Mwanza, United Republic of Tanzania

We conducted a cross-sectional study in northwestern Tanzania (2024) to investigate the prevalence and distribution of trematode parasites (*schistosomes, Fasciola, and paramphistomes*) among snails, humans, and livestock, intending to inform One Health–based control strategies. A total of 2,013 *Bulinus nasutus* and 626 *Lymnaea natalensis* snails were collected from aquatic habitats. Shedding revealed infection rates of 2.98% in *B. nasutus* (schistosomes) and 4.15% in *L. natalensis* (*Fasciola*). Schistosome shedding in *B. nasutus* did not vary significantly by region, waterbody type, or use, whereas *Fasciola* infections in *L. natalensis* were highest in Sengerema district and Kasungamile village. Human participants (668 males, 686 females) provided urine and stool samples. *Schistosoma haematobium* prevalence was significantly higher in males (p=0.0043) and in adolescents aged 12–21 years (p=0.03); *S. mansoni* prevalence was very low. Livestock sampling (828 cattle, 690 goats, 662 sheep) detected *Paramphistomes* (64%), *Fasciola gigantica* (35%), and schistosomes (1%), with significant differences between Mini-FLOTAC and sedimentation techniques (p<2.2e-16). The results indicate that schistosome transmission in humans is primarily linked to *B. nasutus* snails, suggesting the need for interventions to reduce human water contact. The high livestock trematode burden calls for separate, integrated control measures tailored to the animal production system.

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PREVALENCE AND CHARACTERIZATION OF HEPATIC FIBROSIS AMONG INDIVIDUALS LIVING IN A *SCHISTOSOMA JAPONICUM*-ENDEMIC REGION OF THE PHILIPPINES

Susannah Colt[1], Hannah Wu[1], Mario Jiz[2], Ralph Narido Aniceto[2], Veronica Tallo[2], Kirsten d'Hemecourt[1], Jonathan D. Kurtis, MD/PhD[1], Andreas Neumayr[3], Christoph Hatz[3], Jennifer F. Friedman[1], Joachim Richter[3]

[1]Brown University, School of Medicine, Providence, RI, United States, [2]Research Institute for Tropical Medicine, Muntinlupa City, Philippines, [3]Swiss Tropical and Public Health Institute, Basel, Switzerland

Approximately 250,000 deaths are attributed to schistosomiasis annually. The primary cause of severe morbidity and death due to intestinal schistosomiasis is liver fibrosis with consequent portal hypertension and bleeding. *Schistosoma japonicum* causes portal fibrosis (PF) and, uniquely, interseptal fibrosis (ISF). Few if any studies have examined associations between these types of fibrosis and measures of liver morbidity. Individuals with *S. japonicum* infection in Leyte, The Philippines, diagnosed by Kato-Katz, were enrolled and underwent ultrasonographic assessment of the liver, spleen, and portal vasculature, including "danger signs" for bleeding risk. PF and ISF were identified and categorized into characteristic sub-patterns by ultrasound. Image pattern A indicates a healthy liver, and B is a non-specific pattern. Multivariate regression models were used to assess risk factors and liver morbidity measures associated with fibrosis types. Of N=373 participants, the distribution for fibrosis categories was 43 (11.5%) pattern A, 90 (24.1%) pattern B, 48 (12.9%) PF only, 115 (30.8%) ISF only, and 77 (20.6%) combined PF and ISF. Older age was associated with higher odds for PF (OR=3.22, p=0.04), ISF (OR=5.50, p<0.01), and combined (OR=7.85, p<0.0001) compared to pattern A. Male versus female sex was associated with higher odds for combined (OR=6.31, p<0.001). Of the participants classified with danger signs (N=11), all had either PF (18.2%) or combined (81.8%). PF (ß=0.21, p<0.0001), ISF

(ß=0.18, *p*=0.001), and combined (ß=0.20, *p*<0.0001) were associated with a higher portal vein quotient. Combined PF and ISF were associated with higher odds for elevated alkaline phosphatase (OR=5.08, *p*=0.002). This study found specific risk factors for the types of fibrosis uniquely seen with *S. japonicum* infection and distinct liver morbidities associated with fibrosis types.

6820

RESOLUTION OF CLINICAL TRACHOMA SIGNS IN CHILDREN AFTER AZITHROMYCIN TREATMENT: A FOLLOW-UP STUDY IN THE AMAZON

Evelyn R. Munayco-Pantoja[1], Martina Guillermo-Roman[1], Dionna M. Wittberg[2], Jeremy D. Keenan[2], Andres G. Lescano[1]

[1]Universidad Peruana Cayetano Heredia, Lima, Peru, [2]University of California San Francisco, San Francisco, CA, United States

Azithromycin is the standard active trachomatous inflammation—follicular (TF) treatment, but its effectiveness in areas with high TF prevalence but low ocular Chlamydia trachomatis infection, such as the Peruvian Amazon, remains unclear. This study aimed to identify factors associated with post-treatment resolution in the Peruvian Amazon. 17 villages were selected by probability-proportional-to-size sampling for this follow-up study, with ~40 households randomly chosen per village. Children aged 1-9 years were examined for TF via conjunctival observation following WHO guidelines. Their guardians completed a household survey. At baseline, TF-positive children received a single dose of 1mg/kg oral azithromycin. After three months, conjunctival examinations were repeated to assess clinical resolution, or TF-positive children converting to TF-negative. A logistic regression model with cluster-robust standard errors at the household level was fitted, including sub-basin as a fixed effect to account for geographic heterogeneity. Analyses were performed in R version 4.5.3, with statistical significance set at $p < 0.05$. A total of 436 children were enrolled; 351 were TF-positive, of whom 347 received oral azithromycin, while 85 were TF-negative. At 3 months, loss to follow-up was 18.4% (n=64) in the TF-positive group and 31% (n=26) in the TF-negative group. Of the 267 TF-positive participants with follow-up data, the median age was 4 years (IQR: 3-6), 40.4% were male, 61.4% had received antibiotics in the past year, 34.5% had a head of household (HOH) with elementary education, and 85% has a HOH who was a farmer. Clinical resolution occurred in 75 children (28.1%). Resolution was approximately twice as likely in females (AOR = 2.14; 95% CI: 1.21-3.78), and children with a HOH with higher education were five times more likely to resolve compared to those with no education (AOR = 4.96; 95% CI: 1.02-24.23). Limited resolution following azithromycin suggests that antibiotic-based strategies alone may be insufficient, with individual and household factors potentially influencing clinical TF resolution.

6821

ESCHERICHIA COLI CONTAMINATION OF BOTTLED WATER ACROSS REGIONS AND WEALTH QUINTILES IN THE DOMINICAN REPUBLIC

John D. McLennan

University of Calgary, Calgary, AB, Canada

The extent of reliance on bottled water to meet household drinking water needs in the Dominican Republic (DR) is among the highest in Latin America and the Caribbean (LAC). Additionally, based on testing for the presence of Escherichia coli (E. coli) in bottled water samples through the Multiple Indicator Cluster Survey (MICS) program, the DR had the highest prevalence of contamination in LAC countries. This study investigated whether the problem of E. coli contamination of bottled water in the DR is regionally specific or a pervasive problem in the DR, as well as whether it is concentrated among poorer households who may have access to less reliable quality water. Data from the 2019 nationally representative household survey (MICS) in the DR were used. From drinking water source samples from 2,057 households, the prevalence of E. coli contamination in bottled water was 51.6% (95% Confidence Interval [CI]: 48.4, 54.6). Across the 10 geographic/administrative regions, prevalence varied from a low of 41.2% (Cibao Nordeste) to a high of 61.4% (Enriquillo). Across wealth quintiles it ranged from a low of 41.3% among the richest quintile to 59.5% among the poorest quintile. Using the Ozama region as the reference (the large urban region that contains the national capital), Cibao Nordeste was the only region that differed significantly, with lower odds ratios (OR) of E. coli contamination (OR: 0.59 [95%CI: 0.37, 0.93]) based on a logistic regression model that adjusted for household wealth, and an urban/rural classification. In the same adjusted model, households in lower wealth quintiles had higher ORs of E. coli contamination in their bottled water compared to the richest quintile. Although some variation by region and wealth quintile is evident, E. coli contamination is pervasive and prevalent across all regions and wealth quintiles in the DR. Identification of gaps in bottled water processing, management and regulation in the DR is needed and should also include consideration of water stations and other forms of packaged and refilled bottled water which are also increasing in this country as sources of drinking water.

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ENVIRONMENTAL SURVEILLANCE OF *NAEGLERIA FOWLERI* IN SOUTH CAROLINA WATER BODIES

Kennedy Flynn[1], Brandon Hollingsworth[1], Madeleine Meyer Torelli[2], Mary Katherine Lynn[2], Kia Zellars[1]

[1]University Of South Carolina, Columbia, SC, United States, [2]University of South Carolina, Columbia, SC, United States

Naegleria fowleri is a free-living aquatic amoeba and most common cause of primary amoebic meningoencephalitis (PAM) in humans. With an estimated 16 cases per year, PAM has a nearly 97% fatality rate. The majority of PAM cases have been associated with recreational activities in waterbodies, after accidental inhalation of amoeba-contaminated water that reaches the central nervous system. Three fatal cases of this rare infection have been reported in South Carolina within the last decade, including one case in July 2025 from Lexington County. Despite the public health risk, the relationship between environmental conditions and *N. fowleri* concentration is currently unknown. We will sample 15 waterbodies, consisting of a mix of rivers, small lakes, and large lakes, throughout South Carolina (Upstate, Midlands, and Low Country) that are commonly used for recreation. At each waterbody, we will collect 1L of freshwater from high and low recreational use areas once a month from May to September. During visits, we will also measure biotic and abiotic water conditions, including temperature, dissolved oxygen, and pH. We will then determine *N. fowleri* concentration within samples by qPCR. Understanding the environmental conditions that influence the concentration of *N. fowleri* can provide insight into environmental risk as well as determining what is considered an abnormal concentration of amoeba in water. It is essential to determine environmental conditions associated with the concentration of *N. fowleri* in local water bodies to help inform the public, allowing them to take appropriate preventative measures.

6823

WASTEWATER-BASED EPIDEMIOLOGY OF ROTAVIRUS A: A STUDY OF PREVALENCE, INFECTIVITY AND GENETIC DIVERSITY IN GHANA, 2024

Emmanuel Gberbi

University of Ghana, Department of Medical Microbiology, Accra, Ghana

Rotavirus remains a leading cause of acute gastroenteritis in children under five years of age, particularly in Africa, despite the introduction of rotavirus vaccines. Wastewater-based epidemiology (WBE) has emerged as a valuable surveillance approach for monitoring pathogen circulation at the community level, including asymptomatic or clinically underreported cases. This study investigated the prevalence, infectivity, and genetic diversity of rotavirus in wastewater in Ghana during 2024. Wastewater samples were collected from twelve surveillance sites across six regions of Ghana and concentrated using the Polyethylene glycol (PEG) method. Rotavirus was detected by targeting the VP6 gene. The viral infectivity was determined

using integrated cell culture technique, the VP4 and VP7 genotypes were determined and phylogenetically analysed. Out of 122 wastewater samples tested, 68% (83 samples) were positive for rotavirus A. Partial VP7 and VP4 gene sequencing of rotavirus showed that, P[8] (18%) and P[6] (12%) were the predominant P-types. G1P[8] (35%) and G3P[8] (18%) were the most frequently detected G/P combinations. Phylogenetic analysis of rotavirus sequences from Ghana revealed high genetic diversity, as strains grouped with globally circulating lineages from Africa and Asia, indicating widespread dissemination of common rotavirus variants. Infectivity assessment revealed 20% of positive rotavirus were potentially infectious, indicating replication and possible transmission. The high prevalence of human rotavirus A in wastewater suggests ongoing community circulation, potential infectivity underscores the need for targeted preventive interventions and genetic diversity demonstrates the co-circulation in Ghanaian wastewater. These findings highlight the urgent need to integrate WBE into the national surveillance program.

6824

SOCIODEMOGRAPHIC AND WATER, SANITATION, AND HYGIENE (WASH) DETERMINANTS OF DIARRHEAL AND WORMY STOOL IN LARGEST FLOATING URBAN SLUM IN NIGERIA

Babatunde Adewale[1], Hammed Mogaji[2], Abraham Ajayi[1], Oluwabusayo Roleola[3], Adewale Ojogbede[1], Opeoluwa Adewale[4], Stella Smith[1]

[1]Nigerian Institute of Medical Research, Lagos, Nigeria, [2]Federal University Oye-Ekiti, Ekiti, Nigeria, [3]Lagos State University, Lagos, Nigeria, [4]81 Division Military Hospital, Lagos, Nigeria

Informal settlements populations are disproportionately exposed to poor environmental and health conditions. Routine surveillance systems rarely capture the health metrics among those living in these marginalized settings. The study assessed the prevalence of diarrhoea and wormy stool among school-aged children (5-17 years) in Makoko. Interviewer administered structured questionnaires were used to collect sociodemographic, environmental, and hygiene-related information, including self-reported experience of diarrhoea and wormy stool within the preceding three months. Most participants were female (52.1%), aged 5-11 years (56.8%), and their parents had completed secondary education (52.1%), traders (40.7%). Prevalence of diarrhoea and wormy stools was 60.7% and 47.5% respectively. Open defecation was reported by 43.2%, while 28.9% shared toilets, and 78.9% relied on boreholes for drinking water. Higher proportions of wormy stools and diarrhoea were observed among those sharing toilets (38.3% vs 33.5%; p = 0.002 and 0.048). For hygiene behaviours, wormy stool was more common among those who reported nail biting (88.0%, p = 0.045) prior to washing their hands before eating (65.4%, p = 0.021). However, diarrhoea was more prevalent among those who practiced geophagy (82.4%, p = 0.053) and walked barefooted (78.2%, p = 0.001). Our regression model shows that male (OR=1.90, 95% CI: 1.11-3.33, p = 0.02) had higher odds of reporting wormy stool. While those whose parents were civil servants (OR = 3.74, 95% CI: 1.29-12.67, p = 0.02) had higher odds of reporting diarrhoea. Further probes reveal these children of civil servants predominantly use pit (25.0%, p=0.067) and shared latrines (41.7%, p=0.014) compared to other children. The study highlights a high burden of self-reported diarrhoea and wormy stool among school-aged children, with notable associations with hygiene and sanitation practices. These findings underscore the need for targeted interventions focusing on improved hygiene practices, safe sanitation, and behavioural change strategies to reduce enteric infections in underserved population settings.

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WATER, SANITATION, AND HYGIENE (WASH) PRACTICES AND SOIL-TRANSMITTED HELMINTH INFECTIONS AMONG SCHOOL-AGE CHILDREN IN OGUN STATE, NIGERIA: A CROSS-SECTIONAL ANALYSIS

Temitope Michael Ogunbi

Evidence Action Development Initiative Nigeria, Abuja, Nigeria

Soil-transmitted helminth (STH) infections remain a major public health burden in sub-Saharan Africa, disproportionately affecting school-age children. Water, sanitation, and hygiene (WASH) practices are key modifiable risk factors, yet evidence linking school-based WASH behaviors to STH risk in Nigerian settings is limited. This study examines WASH-associated factors and their relationship with STH infection. A cross-sectional survey was conducted among 2,154 primary school students across twenty Local Government Areas in Ogun State, Nigeria. Stool samples were analysed for STH. WASH data were collected via questionnaires aligned with the WHO WASH toolkit covering sanitation access, defecation and urination practices, handwashing, footwear use, and water sources. Multivariable regression with random intercepts at school and LGA levels identified WASH factors independently associated with STH infection. Drinking from unprotected springs was associated with 65 (95% CI: 8.8-486.87, p<0.001) times higher odds relative to government water drums. Improved water sources were associated with 0.4 (95% CI: 0.31-0.66, p<0.001) times the odds compared to unimproved sources. Handwashing with soap after toilet use was strongly protective (OR: 0.03). Wearing footwear to school was associated with 0.2 (95% CI: 0.09-0.35, p<0.001) times the odds of infection. At school level, drinking from unprotected springs had substantially higher odds (OR=560.7, 95% CI: 37.33-8421.42, p<0.001) compared to government drums, and lacking a water source also increased odds (OR=46.2, 95% CI: 3.68-579.39, p=0.002). Having a functional toilet (OR=0.16, 95% CI: 0.06-0.46, p<0.001), handwashing with soap (OR=0.03, 95% CI: 0.004-0.18, p<0.001), and school health education (OR=0.12, 95% CI: 0.02-0.6, p=0.009) were protective. Specific WASH behaviors—open defecation, unprotected water sources, and absence of soap handwashing—are strongly associated with STH among schoolchildren in Ogun State. These findings underscore the need to integrate targeted WASH interventions within deworming programs for sustained STH control in endemic settings.

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PERSISTENT ORGANIC POLLUTANTS IN SMOKELESS TOBACCO FROM UZBEKISTAN, LEVELS PATTERNS AND RISK ASSESSMENT

Jasur Juraev

Kyoto University, Kyoto, Japan

Smokeless tobacco (SLT) products, notably nasvay, are widely used in Uzbekistan, yet the extent and implications of pesticide contamination in these products are not well understood. This study provides the first comprehensive evaluation of persistent organic pollutants (POPs) in SLT across six key regions of Uzbekistan, focusing on both their presence and associated health risks.Twenty-five SLT samples were systematically collected from the Tashkent City, Tashkent Region, Jizzakh, Samarkand, Fergana, and Kashkadarya. Using gas chromatographymass spectrometry, concentrations of organochlorine pesticides including hexachlorocyclohexane isomers, endosulfan, DDT, hexachlorobenzene, toxaphene, dieldrin, and mirex-were quantified. The study documented marked regional variation in SLT contamination, with Jizzakh and Tashkent displaying notably high levels of endosulfan (endosulfan-1 up to 17.92 ng/g, endosulfan-2 up to 27.34 ng/g), while Fergana showed lower overall contamination. The persistence of legacy pesticides such as DDT and hexachlorobenzene underscores ongoing environmental contamination and inadequate regulatory enforcement. Human exposure estimates indicated that hazard quotients and cumulative hazard indices for individual POPs remained below international safety thresholds under modeled scenarios; nonetheless, the frequent detection of highly toxic and banned substances

raises valid public health concerns. These results highlight an urgent need for the development and enforcement of systematic safety standards, routine surveillance, and targeted toxicological risk assessments for SLT products. Regulatory intervention is essential to mitigate chronic health risks among SLT users and reduce the long-term burden of pesticide-related diseases in Uzbekistan.

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ENVIRONMENTAL DNA FOR COST-EFFECTIVE MULTI-PATHOGEN MAPPING IN WATER SOURCES

Daniel Sher[1], Tamar Dikstein[1], Dikla Aharonovich[1], Asrat Meleko[2], Noa Dahan[3]

[1]University of Haifa, Haifa, Israel, [2]NALA, Tel Aviv Yafo, Israel, [3]Tel Aviv-Yafo, TA, Israel

Waterborne diseases cause at least 1.4 million deaths each year, yet there are currently few high-resolution “risk maps” that identify problematic water sources and the pathogens they harbor. Such risk maps could enable targeted interventions and community-level risk mitigation, allowing the population to avoid high risk water sources, and decision-makers to carry out systematic monitoring and treatment. One reason for this gap is that there are many different pathogens and/or disease vectors and hosts, each of which requires a pathogen-specific detection method that is often costly and operationally complex. The presence of DNA from organisms living in and around water bodies (environmental DNA, or eDNA) can be used to determine whether a water body contains pathogens or their vectors, but current molecular methods are often tailored for specific diseases, are not cost effective, and are difficult to deploy in low resource settings. Here, we ask whether amplicon sequencing of common marker or barcode genes such as the small ribosomal subunit (16S/18S) or the cytochrome c oxidase unit 1 (COI) can be developed into a scalable, cost-effective approach for multi-pathogen risk mapping. We systematically tested, using computational methods, whether dozens of commonly-used primers can amplify marker genes from multiple pathogens, and whether the resulting sequences can differentiate between the pathogens and related organisms. We show that some marker genes can specifically identify common pathogens such as *Vibrio cholerae* and *Schistosoma mansoni*, and demonstrate that the latter can be identified in water samples from Southwestern Ethiopia. These findings suggest that broad-range amplicon sequencing with appropriately selected universal primers could enable integrated, multi-pathogen surveillance and support targeted control in endemic settings. Key challenges remain, including sample preservation, cross-contamination, centralized processing, and local workforce capacity.

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POULTRY MANAGEMENT PRACTICES AS INDICATORS FOR EXPOSURE TO ZOONOTIC PATHOGENS: A LONGITUDINAL ANALYSIS WITHIN AND ACROSS HOUSEHOLDS IN BANGLADESH

Elizabeth D. Thomas[1], Ayse Ercumen[2], Angela Harris[2], Abul Kamal[3], Laura H. Kwong[4], Mohammad Atique Ul Alam[3], Zahid Hayat Mahmud[3], Mahbubur Rahman[3], Jesmin Sultana[3], Peter J. Winch[1]

[1]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [2]North Carolina State University, Raleigh, NC, United States, [3]icddr,b, Dhaka, Bangladesh, [4]University of California, Berkeley, Berkeley, CA, United States

Poultry ownership is a source of zoonotic pathogen exposure. Accurately characterizing poultry management practices is important for identifying exposure risks. Little is known about the stability of common poultry exposure measures over time. In rural Bangladesh, we conducted a longitudinal cohort study with 75 households in compounds with domestic poultry. We visited households in two rounds of data collection six months apart; each round had two visits two weeks apart, totaling four visits. At each visit, enumerators checked for free-roaming poultry, poultry housing, and poultry feces piles in multiple locations in the compound, and administered a survey on poultry ownership, nighttime poultry housing practices, interactions with poultry, and poultry feces management. We assessed variation in exposure indicators across visits and rounds at the population level (i.e. compared means across households) and household level (i.e. quantified variability within households). We also calculated reliability estimates to assess if survey responses and observations were stable over four visits. Poultry ownership was a stable household-level indicator across all visits. Flock size was less stable, with significant population-level differences between rounds for chicken and duck flock sizes and poor household-level stability for chickens. Access to a poultry shed outside (i.e., in the courtyard) varied little across visits but whether households kept poultry inside the dwelling at night varied across visits. At the household level, poultry feces indicators were unstable for all locations across and within rounds. At the population level, mean feces counts were significantly lower in Round 2 in the courtyard, kitchen, and veranda, but were stable inside the dwelling. These findings have implications for exposure assessment in similar settings. Repeat measurements for flock size and nighttime poultry housing location are needed to accurately characterize exposure. Household-level poultry feces indicators alone may be unstable proxies for fecal exposure, though population-level feces indicators for some locations may be useful.

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SHAME, DISGUST AND DISCOMFORT: IMPLICATIONS OF LIMITED ACCESS TO WATER, SANITATION AND HYGIENE (WASH) ON QUALITY OF LIFE IN SCHISTOSOMIASIS ENDEMIC COMMUNITIES IN UGANDA AND CAMEROON.

Lazaaro Mujumbusi[1], Lucy Pickering[1], Valerie Makoge[2], Nambusi Kyegombe[3], Rachel Kawuma[3], Margaret Ivy Namukobe[3], Slivesteri Sande[3], Michele Signe[2], Sergi Alonso[1], Derick NM Osakunor[1], Poppy Lamberton[1]

[1]University of Glasgow, Glasgow, United Kingdom, [2]Institute of Medical Research and Medicinal plants Studies, Yaounde, Cameroon, [3]Medical Research Council/Uganda Virus Research Institute & London School of Hygiene and Tropical Medicine, Uganda Research Unit, Entebbe, Uganda

In sub-Saharan Africa, limited access to water, sanitation and hygiene (WASH) remains a major public health challenge. Beyond driving infectious diseases such as gastrointestinal viral and bacterial infections, schistosomiasis and soil transmitted helminthiasis (STH), inadequate WASH access also has broader impacts on people’s wellbeing. While existing research focuses mainly on its biomedical outcomes, the psychosocial consequences remain underexplored. In this study, we adopted a capability approach to investigate the social impacts of limited WASH access and its effects on people’s quality of life in Uganda and Cameroon. A qualitative study was conducted between April and July 2025 in six schistosomiasis-endemic communities. Data were collected using 60 in-depth interviews, 42 focus group discussions, and 20 photo elicitation interviews. Data were coded in NVivo14 and analysed thematically. Limited WASH access negatively impacted psychosocial, economic and mental wellbeing, ultimately shaping people’s quality of life. Lack of adequate sanitation facilities such as latrines and bathrooms, contributed to feelings of shame, loss of respect and dignity, embarrassment before visitors, and low self-esteem. Poorly maintained unclean latrines evoked disgust and promoted open defecation which was associated with discomfort and lack of privacy. In the absence of adequate latrines and bathrooms, participants in Uganda reported open defecation and bathing in lakes, exposing them to physical and environmental risks such as sexual and domestic violence, snake and crocodile bites. These experiences disrupted cultural norms, affected family, community and work relationships. Limited access to safe water was perceived to increase disease transmission, resulting in higher treatment costs and neglect of other basic needs. The impacts of inadequate WASH extend beyond biomedical outcomes, significantly affecting social wellbeing and quality of life. Thus, integrating social dimensions into WASH and schistosomiasis interventions is essential in designing holistic strategies to improve health outcomes and overall wellbeing.

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HAND HYGIENE COMPLIANCE IN THREE REGIONAL HOSPITALS IN BURKINA FASO: FINDINGS FROM A WHO BASED AUDIT

Lucienne Sandwidi[1], Guy Bertand Rouamba[2], Ousmane Badolo[1], W. Félicité Nana[3], James Sarkodie[4], Amos Asiedou[4]

[1]Jhpiego, Ouagadougou, Burkina Faso, [2]Ministry of health, Ouagadougou, Burkina Faso, [3]Ministry, Ouagadougou, Burkina Faso, [4]Jhpiego, Accra, Ghana

Hand hygiene (HH), an essential measure for the prevention and control of healthcare-associated infections (HAIs), remains insufficiently practiced. The objective of our study was to assess knowledge, attitudes, and practices related to hand hygiene in three Regional Hospital Centers (RHCs) in Burkina Faso. A descriptive cross-sectional observational audit was conducted from January to February 2025 in three Regional Hospitals. Data was collected using World Health Organization (WHO) tools. Hand hygiene opportunities were identified according to the WHO *Five Moments for HH* during routine care activities. Systematic non-participant observation of healthcare workers, assessment of infrastructure and consumables, interviews with healthcare workers, and review of communication materials were conducted by trained members of the infection prevention and control IIPC) committees. Kobo Toolbox was used for data collection, and the analysis was performed using the R software. In total, 1,941 hand hygiene opportunities were observed across the three Regional Referral Hospitals (RRHs), with an overall mean compliance rate of 34.11%. The compliance rate by WHO indication was: 31% before touching a patient; 27.8% before an aseptic procedure; 49.8% after risk of exposure to body fluids; 43% after touching a patient; and 29.9% after touching the patient's environment. One hundred percent of RHC had running water, and 100% had alcohol-based hand rub, but it was not available at the point of care. Sixty-five percent of staff were aware of the five indications for hand hygiene. The level of observance of the three hospitals (34.11%) remains below international benchmarks, where average compliance ranges between 40% and 60% according to the WHO. Analysis by hospital revealed marked heterogeneity in performance: the Tenkodogo RHC recorded a compliance rate of 53.70%, comparable to international levels, whereas compliance remained substantially lower at the Gaoua RHC (36.26%) and the Koudougou RHC (20.44%). These findings highlight the need for multimodal interventions to achieve sustained improvement in practices

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ASSESSMENT OF VACCINATION WASTE MANAGEMENT AND PROPOSAL OF A SUSTAINABLE MANAGEMENT MODEL IN THREE REGIONS OF BURKINA FASO

Lucienne Sandwidi[1], Christel Neya Ouedraogo[2], Boureima Ouedrao[1], Annick. R. O Sidibe[3], Romial Sawadogo[4], Anicet G Dahourou[4], Emilie Therese Dama[4]

[1]Jhpiego, Ouagadougou, Burkina Faso, [2]Ministry of health, Ouagadougou, Burkina Faso, [3]Jhpiego, Baltimore, WA, United States, [4]US CDC Burkina Faso, Ouagadougou, Burkina Faso

Accidental needlestick injuries from contaminated sharps pose significant occupational health risks, with transmission probabilities estimated by the World Health Organization at 30% for hepatitis B virus, 1.8% for hepatitis C virus, and 0.3% for HIV. Effective management of vaccination related sharps waste is therefore essential, particularly in low resource settings. This study aimed to assess the status of vaccination waste management in three regions of Burkina Faso and to propose shared management scenarios to improve health safety, environmental protection, and resource efficiency. The Appropriate Disposal of Immunization Waste (ADIW) tool was used to quantify immunization waste, assess disposal practices, and model pooled waste management scenarios based on cost, feasibility, and environmental impact. Regional Ministry of Health workers were trained to support data collection and analysis. In 2024, health facilities serving a population of 278,534 were expected to generate over 2.2 million vaccine vials and approximately 714,000 needles. At the time of data collection, untreated waste stocks were estimated at 25,863 vials and 1.3 million needles. In one region, no health facility had functional infrastructure for vaccination waste treatment. Four shared management scenarios were developed. The estimated average monthly cost per health facility was approximately 20 dollars using local incinerators, compared with about 350 dollars for modern incineration facilities. In several facilities, accumulated waste exceeded annual production levels, indicating prolonged storage and elevated health risks. Vaccination waste management in the study regions faces major operational and infrastructural challenges. Shared management approaches offer a feasible and cost-effective solution to reduce health and environmental risks. Advocacy workshops involving administrative and health authorities were conducted to facilitate adoption of the proposed scenarios.

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WHY SOIL-TRANSMITTED HELMINTHIASIS PERSISTS AFTER 15 YEARS OF MASS DRUG ADMINISTRATION IN RWANDA: MAPPING DIRECT, INTERMEDIATE, AND PREDISPOSING DRIVERS

Jeanne JU Uwizeyimana[1], Eugene Ruberanziza[2], Ladislas Nshimiyimana[3], Jean Bosco Mbonigaba[4]

[1]Heart and Sole Action/Africa- Mwele Malecela Mentorship program (a mentee), Kigali, Rwanda, [2]The END Fund, Kigali, Rwanda, [3]Kigali City, 1, Rwanda, [4]Rwanda Biomedical Center/ Ministry of Health, Kigali/ Rwanda, Rwanda

Despite over 15 years of bi-annual mass drug administration (MDA) with high coverage, some Rwandan districts maintain high soil-transmitted helminth (STH) prevalence. Understanding behavioral, environmental, and structural drivers is crucial for elimination.In 2023, we conducted a population-based baseline assessment in Rubavu District -an area with highly persistent STH infections, enrolling 3,464 participants from 35,080 population. Stool samples were analyzed using duplicate Kato-Katz microscopy for Ascaris lumbricoides, Trichuris trichiura, and hookworm. Immediate, intermediate (environmental), and predisposing (demographic/ structural) drivers were assessed using multilevel logistic regression. Overall STH prevalence was 87.3%, with 51.0% harboring moderate-to-heavy intensity (MHI) infections. School-aged children (5-15 years) had the highest burden (96.2% infected; 72.1% MHI). A. lumbricoides (74.2%) and T. trichiura (73.6%) dominated; hookworm was <1%. Prevalence and intensity varied across villages (78-94%), highlighting local hotspots masked by district averages. Immediate drivers included eating with unwashed hands in agricultural settings (aOR 2.30, 95% CI 1.41-3.73, $p < 0.001$). Intermediate drivers were poor latrine hygiene (aOR 3.41, 95% CI 1.93-6.03, $p < 0.001$), lack of household water treatment (aOR 3.67, 95% CI 2.10-6.43, $p < 0.001$), flies on vegetables (aOR 3.76, 95% CI 1.36-10.43, $p = 0.011$), and water affordability issues (aOR 5.12, 95% CI 1.68-15.62, $p = 0.004$). Predisposing drivers included school-aged children (aOR 4.75, 95% CI 3.40-6.63, $p < 0.001$), long-term residence (aOR 1.56, 95% CI 1.23-1.99, $p < 0.001$).High-coverage bi-annual MDA alone has not eliminated STH in high-prevalence areas. Persistent infection reflects the interaction of behavioral, environmental, and structural drivers. Effective elimination requires intensified MDA, routine drug-efficacy monitoring, targeted WaSH interventions, and behavior change strategies addressing high-risk agricultural exposure. This study provides evidence to guide context-specific STH elimination programs.

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MICROBIAL CONTAMINATION OF SOME VEGETABLES AND IRRIGATION WATER FROM TEN FARMS IN THE ASAMANG COMMUNITY, GHANA

Agnes Acheampong[1], Vanessa Bekota[2], Quinsta Adomah[3]

[1]Ghana Education Service, Kumasi, Ghana, [2]University of Energy and Natural Resources, Sunyani, Ghana, [3]University of Skills Training and Entrepreneurial Development, Kumasi, Ghana

Food safety remains a critical public health issue, especially in areas where poor sanitation and the use of unsafe irrigation water compromise

agricultural practices. This study therefore, assessed the level of microbial and parasitological contamination in selected vegetables and irrigation water collected from ten (10) farms in the Asamang Community, Ghana. Using a cross-sectional study design, a total of 10 irrigation water samples and 32 vegetable samples were collected from 10 farm sites to assess contamination with pathogenic bacteria and parasites. Microbiological analysis of irrigation water identified *Salmonella*, *Shigella*, and *Proteus* species. Notably, *Salmonella* showed markedly high contamination levels (≥240 × 10^3 CFU/mL) in 8 water samples. In vegetables, bacterial loads were also substantial, with *Salmonella* recorded at 141 × 10^3 CFU/mL, particularly in carrots (80 × 10^3 CFU/mL). *Proteus* spp. (55 × 10^3 CFU/mL) were most frequently isolated from cabbage, while *Escherichia coli* (35 × 10^3 CFU/mL) was the least detected among the vegetable samples. Parasitological examination revealed the presence of *Strongyloides stercoralis* (n = 28) and *Ascaris lumbricoides* (n = 23) in vegetable samples. Similarly, irrigation water samples were contaminated with *Ascaris* spp. (n = 14), *Hymenolepis* spp. (n = 8), and *Trichuris* spp. (n = 6), indicating widespread environmental contamination. This study underscores the critical need to strengthen monitoring and management strategies within agricultural systems to enhance food safety and protect consumer health.

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DETECTION AND QUANTIFICATION OF FOODBORNE BACTERIA AND QUANTITATIVE MICROBIAL RISK ASSESSMENT OF *SALMONELLA* IN STREET-VENDED GRILLED CHICKEN SKEWERS IN URBAN BENIN, WEST AFRICA

D. Sylvain Dabadé, O. A. Bassitou Koumassa[1], D. Sylvain Dabadé[1]

Faculty of Agricultural Sciences, University of Abomey-Calavi, Cotonou, Benin

Street-vended grilled chicken skewers are a widely consumed source of affordable animal protein in urban West Africa. Yet, inadequate hygiene and temperature control can elevate the risk of foodborne infections. This study evaluated the microbiological quality of street-vended chicken skewers in urban Benin and examined how hygiene and handling practices affect contamination and consumer exposure. A total of 135 samples were randomly collected from vendors across three major cities at different stages of the selling period to capture realistic exposure scenarios. Hygiene and handling practices were documented through questionnaires and direct observation. Microbiological analyses targeted fecal coliforms, *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella* spp., using conventional culture methods with molecular confirmation. Critical deficiencies were observed: raw chicken was transported unrefrigerated, skewers were held at ambient temperature for ~16 h, and nearly all unsold products (99.3%) were stored at room temperature for ~30 h before resale. Most vendors handled food with bare hands (98.6%), did not wash hands before serving (76.3%), and used the same utensils for raw and cooked products (55.6%). Samples exhibited high contamination: fecal coliforms (96.7%), *E. coli* (61.1%), and *Salmonella* spp. (47.8%). Microbial loads reached 7.3 log CFU/g (*S. aureus*), 6.8 log CFU/g (*E. coli*), and 3.0 log MPN/g (*Salmonella*). A probabilistic microbial risk assessment for *Salmonella* spp. is underway due to its epidemiological significance. These findings underscore the pivotal role of hygiene in street food safety and provide evidence to guide targeted interventions.

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INTRODUCING TAFENOQUINE FOR RADICAL CURE OF *PLAMODIUM VIVAX* MALARIA IN COSTA RICA: EARLY IMPLEMENTATION LESSONS

Jennyffer Gonzalez[1], Yadel Centeno[2], Rodrigo Marin[1], Isaac Vargas[1], Emma Margarita Iriarte[3], Jeniffere Ugalde[1], Leopoldo Villegas[4]

[1]Ministry of Health, San Jose, Costa Rica, [2]Consultant IREM, San Jose, Costa Rica, [3]Inter American Development Bank-IDB, Washington, DC, United States, [4]International Advisor Independent, Silver Spring, MD, United States

Relapses due to dormant *Plamodium vivax* (Pv) hypnozoites remain a major barrier to malaria elimination. Tafenoquine (TQ), a single-dose radical cure, offers a simplified alternative to 7-14-day primaquine regimens but requires quantitative G6PD testing to prevent hemolytic events. In 2024, Costa Rica developed a national operational framework to introduce TQ and quantitative G6PD testing within routine health services, with phased implementation initiated in 2025. Following national rollout on October 1, 2025, a programmatic pilot was implemented in 16 selected health facilities, integrating quantitative G6PD testing and TQ into routine case management through a coordinated implementation matrix across clinical, laboratory, regulatory, and surveillance systems. During the first 6 months, 37 confirmed Pv cases were reported. Among eligible patients aged ≥16 years, 36 (97%) underwent quantitative G6PD testing, demonstrating high feasibility of integrating point-of-care diagnostics into routine services. Of those tested, 17 (47.2%) received chloroquine plus tafenoquine for radical cure following confirmation of adequate G6PD activity. All treatments were administered under directly observed conditions, ensuring full adherence. The introduction of TQ enabled supervised delivery of radical cure within routine services, addressing a major limitation of multi-day primaquine regimens. Operational bottlenecks included initial challenges in supply chain coordination for TQ and diagnostic devices, training laboratory and clinical personnel, and integration into surveillance systems; however, these were resolved quickly, and all participating facilities achieved full operational readiness. These findings demonstrate that integrating tafenoquine and quantitative G6PD testing in routine health systems is feasible at the national level and enables reliable, supervised delivery of radical cure. This experience provides a scalable model for introducing single-dose radical cure in elimination settings and supports broader adoption to accelerate Pv.

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RESISTANCE MARKER INSIGHTS FROM CONTEMPORARY CLINICAL STUDIES FOR NOVEL ANTI-MALARIAL DRUGS

Esther Schmitt[1], Michelle Quinlan[2], Celine Risterucci[1], Zhiyan Qian[1], Xiaojun Zhao[3], Johanne Blais[4], Matthew Fishbaugher[4], Jeremie Lincy[1]

[1]Novartis, Basel, Switzerland, [2]Novartis, East Hanover, NJ, United States, [3]Novartis, Cambridge, MA, United States, [4]Novartis, Emeryville, CA, United States

Data obtained from six studies in patients with uncomplicated malaria and one study in patients with severe malaria, which incorporated both resistance marker analyses and clinical outcomes, provide important insights into emerging patterns. These seven randomized trials, conducted between 2018 and 2025 in various East and West African countries, encompassed more than pediatric and adult 2,000 participants. The investigated resistance markers included *Pfkelch13*, *Pfatp4*, *Pfcrt*, *Pfmdr1*, and *Pfcarl*. For uncomplicated malaria, clinical assessments evaluated monotherapies or combination therapies with oral cipargamin, ganaplacide, lumefantrine, and INE963, using artemether-lumefantrine as the comparator. For severe malaria, intravenous cipargamin was compared to artesunate. The studies assessed the frequency of specific single nucleotide polymorphisms (SNPs) and patient outcomes such as parasite clearance time, slope half-life, new infection and recrudescence rates. Longitudinal data from individual countries and clinical sites improve the understanding of the dynamics of resistance marker frequency and their potential influence

on treatment efficacy. More recently, the cultivation of parasites from clinical samples has been established, enabling a comprehensive functional characterization of *Plasmodium* isolates of interest. In the KARISMA study focused on severe malaria, baseline full gene sequencing of *Pfkelch13* and *Pfatp4* was performed for all patients. Intensive parasite monitoring during intravenous monotherapy enabled a comprehensive analysis of parasite clearance for both artesunate and cipargamin when used without partner drugs. The presence of SNPs within and upstream of the propeller domain in Kelch13 affected parasite clearance rates with intravenous artesunate.

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GENOME AND TRANSCRIPTOME ANALYSES REVEAL DIVERSE MECHANISMS OF ARTEMISININ RESISTANCE IN AFRICAN *PLASMODIUM FALCIPARUM* POPULATIONS BEYOND KELCH13

Michal Kucharski[1], Chanaki Amaratunga[2], Mavuto Mukaka[3], Nicholas Day[4], Arjen M. Dondorp[5], Mehul Dhorda[6], Huy Duong Tien Quang[1], Zbynek Bozdech[7], Umberto D'Alessandro[8], Leon Mutesa[9], Olugbenga Ayodeji MOKUOLU[10], Marie Onyamboko[11], Abdoul Habib Beavogui[12], Abul Faiz[13], Dysoley Lek[14], Samwel Gesase[15]

[1]Nanyang Technological University, Singapore, Singapore, [2]Laboratory of Malaria/NIH, Rockville, MD, United States, [3]Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand, [4]Wellcome-Mahidol-Oxford Tropical Medicine Research Unit, Rajithevi, Thailand, [5]Mahidol-Oxford Trop Medicine Res Unit, Ratchatevi, Thailand, [6]Mahidol-Oxford Research Unit, Bangkok, Thailand, [7]Nanyang Technological Univ, Singapore, Singapore, [8]MRC Unit The Gambia, The Gambia, Gambia, [9]University of Rwanda, Rwanda, Rwanda, [10]University Of Ilorin, Ilorin, KW, Nigeria, [11]Kinshasa Sch of Pub Hlth, Belle Mead, NJ, United States, [12]Minister of Health and University Guinea, Conakry, Guinea, [13]Malaria Research Group, Chittagong, Bangladesh, [14]Cambodia National Malaria Center, Chevy Chase, MD, United States, [15]National Institute of Medical Research, Tanga, United Republic of Tanzania

Resistance to artemisinin in *Plasmodium falciparum* has now independently emerged on the African continent, undermining recent progress in malaria control and elimination. Understanding the genetic basis of this shift is urgent - yet comprehensive genome-wide studies in African parasite populations have been recently lacking.Here we present multi-omics analyses from the Development of Triple Artemisinin Combination Therapies (DeTACT) project, a longitudinal genotype-phenotype association study across eight African countries (September 2020 - January 2024), representing the largest multi-country genomic and transcriptomic survey since the emergence of artemisinin resistance in Africa. We integrated parasite clearance half-life (PC1/2) data with whole genome sequencing and RNA-seq from more than 1,300 isolates to identify genetic determinants of delayed clearance.Multiple resistance mechanisms were identified operating in parallel. By developing novel ultralong amplicon sequencing protocol, we detected gene conversion events in key parasite metabolic genes, pointing to resistance arising through mechanisms beyond de novo point mutations. Genome-wide association analyses identified mutations in RAB cycle gene loci as one of *in vivo* determinants of extended PC1/2, alongside other loci. While kelch13 polymorphisms were associated with prolonged PC1/2 in some countries, this relationship was weaker or absent in others, including high-transmission settings with substantial populations of slow-clearing parasites.Findings presented here reveal a complex, multi-mechanistic architecture of artemisinin resistance in African parasite populations and highlight loci for molecular surveillance and functional validation.

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MEFLOQUINE RESISTANCE COMPROMISES THE EFFICACY OF THE MEFLOQUINE-PIPERAQUINE COMBINATION IN *PLASMODIUM FALCIPARUM*

Camille Roesch[1], Anna Cosson[2], Melissa Mairet Khedim[3], Nimol Khim[3], Nimol Kloeung[3], Sopheakvatey Ke[3], Sreynet Srun[3], Rotha Eam[3], Chanra Kean[3], Chanvong Kul[3], Jean Popovici[3], Rithea Leang[4], Pascal Ringwald[5], Frederic Ariey[6], Romain Coppée[2], Benoit Witkowski[1]

[1]Institut Pasteur de Madagascar, Antananarivo, Madagascar, [2]Laboratoire de Parasitologie-Mycologie, UR ESCAPE, Université de Rouen Normandie, Rouen, France, [3]Pasteur Institute of Cambodia, Phnom Penh, Cambodia, [4]National Center for Parasitology, Entomology, and Malaria Control, Ministry of Health, Phnom Penh, Cambodia, [5]Mekong Malaria Elimination Programme, WHO, Phnom Penh, Cambodia, [6]Service de Parasitologie-Mycologie, Hôpital Cochin, Paris, France

Triple artemisinin-based combination therapies (TACTs) have been proposed to delay the emergence of multidrug-resistant *Plasmodium falciparum* by combining two partner drugs with an artemisinin derivative. Among these, the mefloquine-piperaquine (MQ-PPQ) combination is considered a promising candidate, based on the assumption that simultaneous resistance to both partner drugs would have statistically reduced risk to arise. Here, we evaluated the efficacy and resistance potential of MQ-PPQ using Cambodian clinical isolates with well-characterized resistance profiles. We show that mefloquine resistance is associated with clear increased tolerance to the MQ-PPQ combination, whereas piperaquine-resistant and -sensitive isolates remain susceptible. Prolonged in vitro drug pressure (four months) led to the rapid emergence of MQ-PPQ tolerance, associated with *Pfmdr1* amplification. Mechanistic analyses further highlight a central role for mefloquine, in this study we demonstrate that mefloquine reduces intracellular accumulation of piperaquine in a dose-dependent manner, providing a functional explanation for the observed antagonistic interaction and compromised efficacy of the combination. These findings suggest that MQ-PPQ-based TACTs may be essentially useless, as any emergence of resistance to the combination would be driven solely by mefloquine, which is easily selected via *Pfmdr1* gene amplification. Our results underscore the need to carefully evaluate drug-drug interactions in TACT design and to prioritize combinations with a lower evolutionary potential for resistance.

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PLASMEPSIN II/III COPY NUMBER VARIATION SHAPES METABOLIC AND PHENOTYPIC RESPONSES TO PIPERAQUINE IN *PLASMODIUM FALCIPARUM*

John Kane[1], Aubrey Schall[2], Lisa Checkley Needham[1], Doug Shoue[1], Sydney Gavula[3], Ian Cheeseman[4], Xue Li[5], Ashley Vaughan[6], Timothy Anderson[5], Manuel Llinas[7], Paul D. Roepe[8], Michael Ferdig[9]

[1]University of Notre Dame, Notre Dame, IN, United States, [2]Georgetown University, Washington, DC, United States, [3]Pennsylvania State University, State College, PA, United States, [4]Texas Biomedical Research Inst Genetics, San Antonio, TX, United States, [5]Texas Biomedical Research Institute, San Antonio, TX, United States, [6]Seattle Children's Research Institute, Seattle, WA, United States, [7]Pennsylvania State University, University Park, PA, United States, [8]Georgetown Univ, Washington, DC, United States, [9]Univ of Notre Dame, Dept of Biology, Notre Dame, IN, United States

A mechanistic understanding of the bimodal *in vitro* piperaquine (PPQ) dose-response phenotype in *Plasmodium falciparum* remains incomplete despite its strong association with mutations in *pfcrt* and copy number amplification in *plasmepsin II/III* (*pm II/III*). Given that plasmepsins play a central role in hemoglobin degradation within the parasite digestive vacuole, and that PPQ accumulates within this compartment and perturbs heme detoxification, the bimodal PPQ dose–response phenotype likely reflects alterations in fundamental biological processes alongside drug-specific effects. Defining how *pm II/III* copy number reshapes parasite biology can inform PPQ-based therapies being increasingly adopted in Africa. We used

isogenic PPQ-resistant parasite lines differing only in *pm II/III* copy number to link genotypic variation with integrated phenotypic, metabolomic, and transcriptional responses to PPQ. Across increasing PPQ concentrations, parasites with elevated *pm II/III* copy number exhibited distinct metabolic responses compared to single-copy parasites, characterized by altered abundance of peptides derived from hemoglobin degradation, directly implicating a core biological pathway long associated with plasmepsin function. Integration of these metabolomic and transcriptional data with phenotypic measurements supports a model in which increased plasmepsin abundance enhances the parasite's capacity to sustain hemoglobin digestion and associated metabolic activity under high PPQ concentrations. This points to a possible mechanistic basis for continued parasite survival at high PPQ concentrations, suggesting that altered hemoglobin processing within the digestive vacuole enables parasites to persist in the high-dose conditions that define the second phase of the bimodal response. Molecular dynamics simulations further suggest that PPQ can directly interact with PM II/III, indicating that these proteins may contribute to PPQ response through both modulation of hemoglobin digestion and potential protein–drug interactions within the digestive vacuole.

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INVESTIGATION OF DRUG-RESISTANT *PLAMODIUM FALCIPARUM* COPY NUMBER VARIATION (CNV), LEVELS IN MOZAMBICAN SAMPLES

Suryan Athreya[1], Noah Brown[1], Clemente Da Silva[2], Fatima Nogueira[2], Jennifer Guler[1]

[1]University of Virginia, Charlottesville, VA, United States, [2]Instituto de Higiene e Medicina Tropical, Lisbon, Portugal

Plasmodium falciparum malaria is one of the deadliest infectious diseases in the world, but like most, disproportionately affects the developing African countries like Mozambique. The rate of malaria cases in Mozambique is among the highest in endemic countries. The most common antimalarials used in Mozambique are Artemisinin Combination Therapies (ACT) which in addition to Artemisinin, need an effective partner drug like lumefantrine, piperaquine, and amodiaquine in order to succeed. However, mutations such as copy number variations (CNVs), which increase the number of dosage-sensitive resistance-genes, subsequently drive multidrug resistance to ACTs. In order to quantify rates of CNVs in Mozambique, we previously designed a novel quadruplex digital-droplet PCR (ddPCR) assay. The medium-high throughput assay is reliable, cost-effective, and sensitive, which makes it uniquely suited to study CNV levels in clinical samples. Our prior study detected a 2% CNV rate in ACT-resistance loci *pfpmp2*, *pfpmp3*, and *pfmdr1*. However, this study only assessed 279 samples from 3 out of 11 regions, which represented a limited view of Mozambican infections. Here, we expanded the use of the ddPCR assay to 450 additional Mozambican samples from 4 new regions. These regions, Sofala, Tete, Inhambane, and Gaza, border the previous three regions and represent roughly 22% of the total Mozambican population. Similar to our previous study, we detected a low 1% CNV rate across the Gaza and Inhambane regions. The results demonstrate CNVs in the *pfpmp3* and *pfmdr1* loci, which suggests resistance to ACTs. Our larger-picture study on Mozambican samples now suggests a nearly country-wide, low level of CNVs that confer resistance to ACT partner drugs. Such uniformity over 700 combined samples is a key insight in a continued fight to monitor the spread of *P.f.* drug-resistance across sub-Saharan Africa. As such, our findings support sustained genetic monitoring with a highly reliable assay, as continued research may reveal the necessity of novel antimalarial drugs and disease-prevention tactics.

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EMERGENCE OF A NOVEL *PLASMODIUM FALCIPARUM* CRT DUPLICATION IN UGANDA ASSOCIATED WITH CHLOROQUINE RESISTANCE MUTATIONS

Nicholas J. Hathaway[1], Thomas Katairo[2], Jacquelyn Ly[1], Stephen Tukwasibwe[2], Francis Ddumba Semakuba[2], Bienvenu Nsengimaana[2], Bryan Greenhouse[1], Isaac Ssewanyana[2], Philip Rosenthal[1], Jessica Briggs[1], Melissa Conrad[3]

[1]University of California, San Francisco, San Francisco, CA, United States, [2]Infectious Diseases Research Collaboration, Kampala, Uganda, [3]John Hopkins University, Baltimore, MD, United States

Mutations within the *Plasmodium falciparum* (Pf) chloroquine resistance transporter (crt) gene mediate drug resistance. These mutations may incur fitness costs, and populations revert to wild type (WT) without drug pressure. The crt K76T mutation, the key mediator of chloroquine resistance, reverted to nearly 100% WT in Uganda by 2020. Copy number variation (CNV) is frequent in Pf, is frequently associated with drug resistance, and may help overcome fitness costs if one copy remains WT while the other carries resistance mutations. CNV in crt was previously observed in West Africa and Laos. Using MAD4HatTeR targeted amplicon sequencing from 8,500 Ugandan samples (2023-2024), we identified crt duplication via read depth analysis and within-sample allele frequencies (WSAF) of ~50/50% in otherwise monoclonal samples. We identified 49 samples with likely crt duplication, with high prevalence (~40-60%) in the West Nile region (near S. Sudan, where artesunate-amodiaquine is first-line), and a nationwide prevalence of 2%. Of these, one isolate contained two WT crt copies, while the remaining 48 (98%) had one WT copy and one copy with the CVIET variant. Among the mutant copies, all but one also encoded H97L. 13 of the CVIET+H97L isolates contained A220S; phasing to a specific copy was not possible with short-read data. Notably, among all samples carrying crt mutations, 72% with K76T and 94% with H97L occurred in crt-duplicated isolates. Whole-genome sequencing confirmed crt duplication and identified a novel ~70kb duplicated region, with breakpoints distinct from the previously described 23kb tandem duplication seen in isolates from West Africa and Laos, suggesting independent emergence. The high prevalence of resistance mutations in duplicated samples implicates CNV as a driving force in the reappearance of K76T and the emergence of H97L in Ugandan parasites, potentially threatening the efficacy of drug combination therapies containing amodiaquine. Phenotypic characterization is currently ongoing.

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IMPACT OF *PLASMODIUM FALCIPARUM* RESISTANCE-ASSOCIATED GENOTYPES ON MALARIA PARASITE CLEARANCE AND PROTECTION FROM INFECTION: A DOUBLE-BLINDED, PLACEBO-CONTROLLED, RANDOMISED TRIAL OF SULFADOXINE-PYRIMETHAMINE AMONG ASYMPTOMATIC CHILDREN 3-5 YEARS OLD IN ZAMBIA

Rosario Martinez-Vega[1], Andria Mousa[2], Jonathan Gwasupika[3], Enesia Chaponda[4], Victor Daka[5], Sydney Mwanza[6], Mukuka Chipunga[5], Ana Chopo Pizarro[7], Harparkash Kaur[8], Charlotte Eddis[9], Lucy C. Okell[10], Emma Filtenborg Hocke[11], Louise Wellmann Thomsen[12], Helle Hansson[12], Michael Alifrangis[13], Khalid Beshir[14], Roland Gosling[14], Caroline Roper[15], Colin Sutherland[16], Mike Chaponda[3], R. Matthew Chico[17]

[1]London School of Hygiene and Tropical Medicine, London, United Kingdom, [2]Imperial College London, London, United Kingdom, [3]Tropical Diseases Research Centre, Ndola, 8, Zambia, [4]National Malaria Control Center, MOH Zambia, Seattle, WA, United States, [5]Tropical Diseases Research Centre, Ndola, Zambia, [6]National Health Research and Training Institute, NDOLA, 8, Zambia, [7]London School of Hygiene & Tropical Medicine, LONDON, United Kingdom, [8]London Sch of Hygiene & Trop Medicine, London, United Kingdom, [9]Population Services International, Washington, DC, United States, [10]Imperial College, London, United Kingdom, [11]University of Copenhagen, Copenhagen, Denmark, [12]København N, 84, Denmark, [13]Centre for Medical Parasitology,

Copenhagen, Denmark, [14]LSHTM, London, United Kingdom, [15]London Sch of Hygiene & Tropical Medicine, London, United Kingdom, [16]London School of Hygiene & Tropical Medicine, London, United Kingdom, [17]London School of Hygiene & Tropical Medicine, Austin, TX, United States

The World Health Organization recommends perennial malaria chemoprevention (PMC) for asymptomatic children in high transmission settings with sulfadoxine-pyrimethamine (SP) being the most widely administered drug. However, widespread resistance to *Plasmodium falciparum* genotypes, particularly *pfdhps* K540E in East Africa, may limit its efficacy. We conducted a two-arm, parallel, double-blind, placebo-controlled trial among asymptomatic children between 3-5 years in Zambia. For seven days, children in the active control arm (n=200) received artesunate (AS) and then placebo SP, whereas the intervention arm (n=400) received placebo and given SP. Thereafter, children were followed 63 days and provided samples for microscopy and qPCR. We used Kaplan-Meier and Cox regression methods to assess time to parasite clearance among Day 0 positives and, separately, 28-day protective efficacy (PE) among Day 0 negatives. By Day 28, initial unadjusted parasite clearance among SP recipients was 73.0% by microscopy (95% CI: 61.5-82.3) and 91.7% by qPCR (95% CI: 81.6-97.2), with no evidence of genotype effect (p =0.760 and p=0.277, respectively). Sustained unadjusted clearance was 30.9% by microscopy and 33.3% by qPCR, with no significant genotype differences (p=0.339 and p=1.000). Clearance estimates will be adjusted using molecular correction. Among participants free of malaria symptoms on Day 0, SP did not provide significant PE against new symptomatic infections (PE= -64.2%; p=0.606), nor new infections by qPCR (PE= 10.5%; p=0.536) whereas a significant moderate protection was observed against microscopy-detected infections (PE= 34.5%, 95% CI: 8.7-57.8; p=0.019). At Day 28, PE was higher against the wildtype 540K compared to the mutant 540E for both microscopy (91.3% vs 20.5%; p=0.047) and qPCR (82.0% vs 6.2%; p=0.042). The high prevalence of 540E mutation in East Africa (88.0% in the trial site) limits the population-level impact of SP-based chemoprevention, supporting the need for alternative antimalarials for PMC.

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MOSQUITO XENOSURVEILLANCE TO ELUCIDATE THE ECO-EPIDEMIOLOGY OF EASTERN EQUINE ENCEPHALITIS VIRUS

Jonathan Karisa, Douglas Brackney, Philip Armstrong, Goudarz Molaei

The Connecticut Argicultural Experiment Station, New Haven, CT, United States

In the northeastern U.S., Eastern equine encephalitis virus (EEEV) is maintained in an enzootic cycle involving *Culiseta melanura* mosquitoes and passerine birds. Historically, human disease outbreaks occurred sporadically; however, since 2003, EEEV has resurged and expanded into new regions. The factors driving this sustained resurgence remain poorly understood, including the routes of virus introduction, maintenance, and spillover. In this study, we use xenosurveillance, a novel complementary approach, to determine host identity, host immunity, and active viral infection from a single mosquito abdomen, thereby better understanding the eco-epidemiological drivers of virus transmission. Mosquitoes were collected in six EEEV foci in Connecticut by aspirating them from resting boxes. The mosquitoes were morphologically identified, and engorged *Cs. melanura* were screened for blood-meal hosts using PCR targeting the mitochondrial cytochrome b gene, followed by Sanger sequencing. Screening for EEEV neutralizing antibodies was performed using indirect ELISA. Mosquito infection status was determined using cell culture and RT-qPCR assays. A total of 1,342 *Cs. melanura* were processed for blood-meal hosts; these mosquitoes obtained blood meals mostly from American robins (14.0%), black-capped chickadee (10.6%), yellow-billed cuckoo (9.50%), and red-eyed vireo (9.32%). Our study reported EEEV seroprevalence of ~13.0% (175/1342), detected in passerine birds. Moreover, EEEV was isolated in ~0.5% (7/1342) of the abdomens tested. This study shows that xenosurveillance can simultaneously identify host species, host immunity, and active infections from a single mosquito blood meal. The findings confirm that *Cs. melanura* predominantly feeds on passerine birds, and the presence of EEEV antibodies in these hosts coincides with viral detection in both birds and mosquitoes, highlighting their role in transmission. Overall, this approach provides valuable insights into the eco-epidemiological drivers of EEEV and enhances the ability to monitor its seasonal introduction and amplification within transmission foci.

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IDENTIFYING INTERFACES FOR HIGH PRIORITY AND EMERGING VIRUSES IN LUZON, PHILIPPINES

Neil Mittal[1], Marana Rekedal[2], Mary-Glazel Noroña[3], Jairue A. Café[3], Catalino S. Demetria[4], Kirk J. Taray[5], Jezryl Jaeger L. Garcia[5], Jozette G. Hisu-an[5], John Christian D. Teñoso[6], Samantha L. Magsanoc[5], Hazel R. Cruz[4], Sophie A. Borthwick[7], Dolyce HW Low[7], Phillip Alviola[5], Mary Grace B. Dacuma[5], Fedelino F. Malbas[4], Gavin J. Smith[7], Eric D. Laing[2]

[1]Henry Jackson Foundation/Uniformed Services University of the Health Sciences, Bethesda, MD, United States, [2]Uniformed Services University of the Health Sciences, Bethesda, MD, United States, [3]Research Institute for Tropical Medicine, Manila, Philippines, [4]Research Institute for Tropical Medicine, Alabang, Muntinlupa City, Philippines, [5]University of the Philippines Los Baños, Los Baños, Laguna, Philippines, [6]University of the Philippines Diliman, Muntinlupa City, Philippines, [7]Duke-NUS Medical School, Singapore, Singapore

The Philippines has experienced historical zoonotic and epizootic outbreaks of Nipah virus (NiV), Reston virus (RESTV), and porcine epidemic diarrhea coronavirus (PEDV). We recently completed an investigation of wildlife sources of NiV in the Philippines, identifying species of flying foxes as potentially important virus reservoirs. Expanding upon that first study, we conducted a two year temporal and spatial analysis for co-circulation of Nipah virus and selected Asiatic filoviruses such as RESTV and Mengla virus (MLAV) in flying foxes and rosette bats (*Rousettus amplexicaudatus*), and evidence of spillover of NiV, RESTV, and coronaviruses in pigs collected at abattoirs in towns nearby the bat roosts, and humans residing within 10 kilometers of the roosts. Swabs were processed for nucleic acid detection by viral family consensus PCR, and blood specimens were tested for the presence of antibodies reactive with the attachment glycoproteins of NiV and related henipaviruses, filoviruses, and selected coronaviruses of interest such as PEDV and canine coronavirus-HuPn virus. The detection of binding and neutralizing antibodies flying foxes followed an enzootic seasonal pattern over 24 months, implying an episodic shedding potential for NiV spillover. Additionally, we estimated 3-5% seroprevalence for orthoebolaviruses and MLAV in flying fox and rosette bat populations. However, we did not detect any NiV infection histories in any of the blood specimens from pigs or humans. Interestingly, in samples collected from pigs, clustering analyses identified low magnitude antibody levels against Menangle virus in 2% (13/617), and moderate magnitude antibody levels against RESTV in 3% (18/617) of the blood specimens collected from pigs. We found no nucleic acid or serologic evidence of infection by swine acute diarrhea coronavirus, PEDV, henipaviruses or filoviruses in samples collected from humans. The completion of risk-based outbreak models serves to limit uncertainty and improve spillover prevention.

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MOLECULAR CHARACTERIZATION OF ORTHONAIROVIRUSES DETECTED IN TICKS IN SENEGAL

Mignane Ndiaye[1], Idrissa Dieng[2], Aminata Badji[1], Moufid Mhamadi[1], Arfang Diamanka[3], Alioune Gaye[1], Ngor Faye[4], Mawlouth Diallo[1], Oumar Faye[1]

[1]Institut Pasteur de Dakar, Dakar, Senegal, [2]Dakar, DK, Senegal, [3]Cheikh Anta Diop University, Dakar, Senegal, [4]Dakar, Senegal

Tick-borne viruses (TBVs) represent emerging and re-emerging threats to human and animal health. Many are potentially zoonotic and circulate across numerous regions worldwide, including Africa, where they remain under-surveilled despite their epidemiological and socio-economic impact. In the absence of effective vaccines or treatments, an integrated approach combining entomological, animal, molecular, and genomic surveillance is

essential to better understand and prevent these risks. In Senegal, regular nationwide surveillance of priority arboviruses, including Crimean-Congo hemorrhagic fever virus (CCHFV), is conducted among febrile patients through the 4S network. To extend this surveillance within a One Health framework, tick and serum collections were carried out in 2021 and 2022 across eight localities in the northern, central, and southern regions of the country, targeting cattle, sheep, and goats. After taxonomic identification, tick pools were screened by RT-PCR targeting various TBVs, and positive samples were further characterized using next-generation sequencing (NGS). A total of 6,135 ticks, belonging to 11 species and four genera (*Hyalomma*, *Amblyomma*, *Rhipicephalus*, and *Boophilus*), were collected. CCHFV was detected in 54 pools across all four genera (predominantly *Hyalomma*) from seven of the sampled localities. In addition, Dugbe virus (DUGV) was identified in 10 pools of *Amblyomma variegatum* collected in southern Senegal. Phylogenetic analyses revealed the co-circulation of CCHFV genotypes I, II, III, IV, and VII, with evidence of reassortment events and close relatedness to West African and European strains. In contrast, DUGV strains exhibited greater genetic stability. The concurrent detection of these TBVs and the subsequent emergence of human CCHFV cases in previously unexpected areas highlight the relevance of the One Health approach and early detection of viruses for warning and epidemic preparedness.

6846

IMPACT OF 2019 BRUMADINHO MINING DISASTER ON ARBOVIRAL INCIDENCE: A COMPARATIVE INTERRUPTED TIME SERIES ANALYSIS IN BETIM AND BRUMADINHO, BRAZIL (2017-2023)

Sri Harsha Soma[1], José A. Ferreira[2], Uriel Kitron[3], Jessica K. Fairley[3]

[1]Rollins School of Public Health, Emory University, Atlanta, GA, United States, [2]School of Medicine, Unifenas University, Belo Horizonte, Brazil, [3]Emory University, Atlanta, GA, United States

Environmental and climatic factors impact the transmission of dengue, chikungunya (CHIKV), and Zika viruses, yet studies on their combined impact with anthropogenic disasters remain limited. This study utilized an ecological quasi-experimental design to analyze the consequences of the January 2019 failure of the Córrego do Feijão dam in Minas Gerais, Brazil, which released 9.7 million m^3 of iron ore tailings with heavy metals into the Paraopeba River, increasing turbidity and creating stagnant pools. Dengue incidence in Brazil surged by 442% over the last two decades, and in 2024, there were nearly six million arboviral cases and 4,000 deaths. Using Interrupted Time Series (ITS) and Controlled Interrupted Time Series (CITS) analyses of SINAN data (2017–2023), this study compared disaster-exposed Betim and Brumadinho with the non-exposed control, Santa Luzia (similar size). The dependent variable was monthly cases; Poisson regression models were adjusted for temperature, precipitation, NDVI, and seasonality. Results showed a significant immediate rise in arboviral incidence from February 2019 to April 2019: Betim showed an IRR = 267.86 (95% CI: 30.57–2347.42) and Brumadinho an IRR = 33.80 (95% CI: 3.20–356.67). Compared to Santa Luzia, affected municipalities showed statistically significant differences immediately post-disaster, with Betim at an IRR = 41.14 (95% CI: 3.88–436.39) and Brumadinho at an IRR = 32.24 (95% CI: 3.71–280.21). No clear evidence of a sustained long-term change in incidence patterns was observed. Environmental covariates such as maximum temperature and higher NDVI played a major role in the rise of arboviral incidence. Inclusion of these covariates alongside seasonal adjustment improved model fit (Pseudo R^2: 0.40 to 0.80). This study showed an immediate rise in arboviral cases in affected municipalities after the disaster, not explained by regional trends. These findings highlight the impact of anthropogenic environmental changes on arboviral infections and provide a robust basis for developing public health preparedness in areas where the effects of climate change and industrial disasters converge.

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ECOLOGICAL FACTORS FACILITATING THE SPREAD OF OROPOUCHE VIRUS OUTSIDE THE AMAZON: A BAYESIAN SPATIOTEMPORAL ANALYSIS OF THE 2024-2025 OROPOUCHE OUTBREAK IN CEARA, BRAZIL

Caroline G. Tangoren[1], Macleidi Varnier[2], Rebeca de Souza Oliveira[3], Elizabeth L. Druschel[4], Higor S. Monteiro[5], Antonio S. Lima Neto[3], Marcia C. Castro[6]

[1]Department of Epidemiology, Harvard T. H. Chan School of Public Health, Cambridge, MA, United States, [2]Graduate Program in Remote Sensing, Federal University of Rio Grande do Sul, Porto Alegre, Brazil, [3]Ceará State Health Secretariat, Fortaleza, Brazil, [4]Department of Environmental Health, Harvard T. H. Chan School of Public Health, Cambridge, MA, United States, [5]Department of Physics, Federal University of Ceará, Fortaleza, Brazil, [6]Department of Global Health and Population, Harvard T. H. Chan School of Public Health, Boston, MA, United States

Since 2023, Latin America has been experiencing an unprecedented outbreak of Oropouche fever. In contrast to Oropouche's earlier concentration in the Amazon rainforest, substantial outbreaks have occurred in new geographies. However, transmission outside the Amazon is very heterogeneous, and finescale ecological drivers of transmission remain poorly understood. We combined Oropouche testing data from the Ceará State Health Secretariat, georeferenced to each individual's residence, with peridomicile ecological data to identify environmental risk factors and areas at high risk of future outbreaks. To investigate the role of a potential breeding habitat for the *Culicoides paraensis* vector, we built a remote-sensing model of banana crops with 10m resolution using Google Satellite Embeddings. Additional data included MapBiomas landuse classes, elevation, water bodies, precipitation, and temperature. We fit a Bayesian hierarchical spatiotemporal logistic model using INLASPDE to estimate associations between ecological factors and Oropouche positivity, adjusting for age, sex, and seasonal effects. Four-week cumulative precipitation and age exhibited strong, non-linear associations with OROV positivity. Females were also more likely to test positive than males (Odds ratio: 1.52, 95% Credibility interval: 1.13-2.04). The proportion of the peridomicile area with banana crops showed a positive association that did not reach statistical significance (OR 1.48, 95% CrI: 0.99-2.30). Based on this model, we identify previously unaffected areas in Ceará state whose local ecology would be suitable for Oropouche transmission. Ongoing serological surveys will provide additional data to investigate these trends. Overall, our data suggest that highresolution geostatistical surveillance can identify submunicipal hotspots and help target vector control and outbreak response in rapidly expanding arboviral epidemics.

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CRIMEAN-CONGO HAEMORRHAGIC FEVER IN AFGHANISTAN, 2022-2025: TRANSMISSION ROUTES, NOSOCOMIAL RISK, AND RIBAVIRIN EFFICACY

Siyu Chen

Cornell University, Ithaca, NY, United States

Background. Afghanistan's largest recorded CCHF epidemic (2024-2025) exposed critical gaps in understanding transmission pathways, healthcare worker (HCW) nosocomial risk, and ribavirin efficacy representing three interdependent determinants of outbreak containment and case fatality. Methods. Retrospective cohort of 737 laboratory-confirmed CCHF patients across six tertiary hospitals (2022-2025). Multivariable Cox regression stratified by WHO severity estimated ribavirin efficacy, adjusting for age, sex, hospital, comorbidity, residence, tick exposure, occupation, and symptom-onset-to-admission delay. HCW nosocomial clustering was tested by permutation analysis (1,000 iterations) comparing observed concurrent patient load during each HCW's exposure window to a null distribution. Transmission pathways were characterised by occupational and tick-exposure profiles. Results. CFR was 27.7% (204/737). Ribavirin reduced mortality by 39% in severe CCHF only (HR 0.61, 95% CI 0.38-0.99; p=0.047); older age dominated in mild disease (HR 1.47/10 years; p=0.016). Direct animal contact — not tick bites (8.7%) — was the primary

transmission route: livestock handlers (26.2%), butchers (10.0%), and household meat preparers (19.4%) comprised the majority of cases. Of 19 infected HCWs (2.6%), 94.7% occurred during concurrent CCHF admissions (median 8 co-admitted patients, IQR 3-14; permutation p=0.012), confirming outbreak-driven nosocomial accumulation over discrete patient-to-HCW events. In conclusion, animal contact drives CCHF acquisition; outbreak volume drives nosocomial risk; and ribavirin benefit is severity-specific. Together these findings reframe prevention, IPC, and treatment prioritisation for CCHF in resource-limited settings.

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MAPPING ARBOVIRAL EXPOSURE THROUGH RESIDUAL CLINICAL SAMPLES: EVIDENCE OF SPATIAL HETEROGENEITY

Ana Pierina Osorio-Medrano[1], **Abigale Proctor**[2], Madeline Kernahan[3], Jonathan Bashor[4], Samantha Lopez[2], Jesus A. Ku-Cachon[1], Karina Jaqueline Ciau-Carrillo[1], Jose Reyes Canche-Pech[5], Leticia Chin-Hau[5], Maria Lopez-Novelo[5], Daniel Canul-Canul[1], Gloria Ramirez-Ospitia[1], Georgina Cardenas-Perez[1], Gloria Barrera-Fuentes[1], Norma Pavia-Ruz[1], Pablo Manrique-Saide[1], Azael Che Mendoza[6], Julissa Guzman-Hernandez[7], Laura Conde-Ferraez[1], Guadalupe Thomas Ayora-Talavera[8], James Earnest[9], Daniel Limonta[9], Christopher N. Mores[2], Henry Nelson Puerta-Guardo[10]

[1]Autonomous University of Yucatan, Merida, Mexico, [2]George Washington University, Washington, DC, United States, [3]Arlington, VA, United States, [4]George Washington University - Mores Lab, Washington, DC, United States, [5]Merida Biomedical Clinical Laboratories, Merida, Mexico, [6]Xalapa, Mexico, [7]Yucatan Department of Health, Merida, Mexico, [8]Merida, Mexico, [9]Orlando, FL, United States, [10]Universidad Autonoma de Yucatan, Merida, Mexico

Aedes-borne viruses (ABVs) such as dengue (DENV), Zika (ZIKV), and chikungunya (CHIKV) remain a major public health concern across tropical and subtropical regions worldwide. In Mexico, particularly in the Yucatan peninsula, these viruses continue to circulate endemically, with annual seasonal outbreaks and repeated waves of arboviral transmission within urban areas, known as *hotspots*. Despite the immunological footprint left by successive outbreaks, the spatial distribution of ABV exposure within urban areas of Merida remains poorly characterized. Here, a seroepidemiological survey was conducted using residual plasma samples (n=542 individuals, aged 2 to 97 years) collected during routine clinical testing in Merida. Samples were tested using the Chembio DPP® ZCD rapid diagnostic test to detect IgM and IgG antibodies against DENV, ZIKV, and CHIKV. Anonymized metadata, including sex, age, and residential addresses, were utilized for georeferenced mapping analyses across the city to visualize spatial patterns of arboviral immunity. Overall, IgM seroprevalence was 10% for DENV, 2.8% for ZIKV, and 6.8% for CHIKV, while IgG seroprevalence reached 41.3%, 22%, and 11%, respectively. Despite higher sampling density in northern Merida, a *non-hotspot* area, markedly elevated IgM and IgG seropositivity was observed in *hotspot* areas, particularly in the central and southern sectors. This study demonstrates persistent spatial heterogeneity in ABV transmission across Merida and identifies substantial immunity gaps, especially for re-emerging ABVs currently driving major outbreaks in the Americas, underscoring Merida's vulnerability to future outbreaks. Geotagged serosurveys using residual clinical samples represent a scalable and complementary approach to existing virologic and syndromic surveillance systems by identifying where populations have previously been infected. Such information is essential for guiding risk mapping aiming to geographically targeted interventions under potential ABV outbreak scenarios.

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CHARACTERIZATION AND COMPARISON OF THE VIRAL COMMUNITIES OF *AMBLYOMMA MIXTUM* AND *IXODES* CF. *BOLIVIENSIS* FROM COSTA RICA AND PANAMA

Luis Enrique Chaves-González[1], Tatiana Murillo-Corrales[1], Sarah Temmam[2], Víctor Montenegro[3], Sergio E. Bermúdez[4], Diana Rojas-Araya[1], Nolwenn Dheilly[2], **Adriana Troyo**[1]

[1]Universidad de Costa Rica, San Jose, Costa Rica, [2]Institut Pasteur, Paris, France, [3]Universidad Nacional, Heredia, Costa Rica, [4]Instituto Conmemorativo Gorgas de Estudios de la Salud, Panamá, Panama

Ticks are known vectors of emerging and reemerging pathogens, including viruses. Although tick virome analyses have allowed for virus discovery in ticks from North America, Europe, and Asia, research on tick-associated virus (TAV) diversity in Latin America is limited to a few tick species in Mexico, Colombia, and Brazil. In Central America, TAVs and their potential impact on public health is unknown. This work aimed to characterize and compare the viral communities of *Amblyomma mixtum* from Costa Rica and *Ixodes* cf. *boliviensis* from Costa Rica and Panama. Questing and host-feeding ticks were collected (four sites in Costa Rica and one in Panama), and 26 tick pools were subjected to RNA extraction, reverse transcription, and Next Generation Sequencing. Microseek was used for contig assembly and virus taxonomic assignation. Alpha diversity (Chao1, Shannon, Simpson, and Pielou indices) and beta diversity (ordination, PERMANOVA, and ALDEx analyses) were assessed at the family level across tick species, collection sites, and collection sources. Overall, virus family richness was greater in *I.* cf. *boliviensis* than *A. mixtum*, and diversity was greater in questing than host-feeding ticks. Ticks from Costa Rica presented overlaps in community structure, while ticks from Panama clustered separately. Notably, viral communities from questing ticks differed markedly from those of host-feeding ticks. Differentially abundant families were identified across variables, including *Partitiviridae, Flaviviridae,* and *Botourmiaviridae* in *A. mixtum,* and *Nairoviridae, Phenuiviridae, Rhabdoviridae,* and unclassified *Bunyaviricetes* in *I.* cf. *boliviensis*. Overall, results show that viral community diversity and composition in *A. mixtum* and *I.* cf. *boliviensis* differed by tick species and are influenced by environmental conditions and tick ecology. Further research is required to identify the detected viral contigs to the species level through genome assembly and elucidate the role of these ticks as putative vectors. To the best of our knowledge, this is the first study aiming to characterize TAV diversity in Central America.

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SEROLOGICAL EVIDENCE FOR CO-CIRCULATION OF DENGUE, CHIKUNGUNYA, AND ZIKA VIRUSES IN DENGUE NS1-NEGATIVE FEBRILE PATIENTS IN BANGLADESH

Afrida Tabassum Trina, Md Fahad Zamil, Anamul Hasan, Mohammad Sharif Hossain, Mohammad Shafiul Alam

icddr,b, Dhaka, Bangladesh

The presence of Dengue, Chikungunya, and Zika viruses is increasingly recognized as a growing public-health concern in Bangladesh, as transmission by a single mosquito genus, *Aedes*, heightens the risk of simultaneous circulation and overlapping outbreaks. A substantial number of cases may remain undetected due to similar clinical presentations and the reliance on rapid diagnostic tests that primarily detect dengue. In contrast, chikungunya and Zika virus infections remain largely underdiagnosed due to the lack of targeted surveillance and limited availability of diagnostic kits. In this study, during routine dengue testing conducted among the suspected patients in 2024 from three collection centers of icddr,b in Dhaka , a total of 427 Dengue NS1 RDT-negative samples were further analyzed to identify any seropositive infections that may have been missed out due to delayed testing after the onset of fever. Based on the availability of each IgM ELISA kits, these samples were randomly selected to assess recent exposure for DENV (DENV Detect™ IgM Capture ELISA), ZIKV (ZIKV Detect™ 2.0 IgM Capture ELISA), and CHIKV (Human Anti-Chikungunya Virus IgM ELISA Kit) separately. Following the final analysis, DENV IgM was reactive in 3 out of 64 (4.6 %) samples,

CHIKV IgM was detectable in 47 out of 200 (23.5%), and presumptive ZIKV IgM was detected in 30 out of 299 (10.03 %) of samples, with an additional 17 samples (5.65%) showing reactivity suggestive of other flaviviruses. Cases were concentrated mainly during the months of August to December. All IgM-positive cases showed a median of 3 days, (IQR: 2-3 days) from time of onset of fever onset to testing. As IgM positivity indicates recent infection, these findings demonstrate that a considerable number of arboviral infections remain undetected through routine dengue diagnostics and confirm the co-circulation of major arboviruses. The results highlight the need for expanded serological surveillance to improve case detection, strengthen outbreak response, and better characterize population-level exposure to multiple arboviral infections.

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SHIFTING AGE PROFILE OF MALARIA BURDEN IN NIGERIA (2016-2025): A RETROSPECTIVE LONGITUDINAL ANALYSIS

Yahaya Yaqub[1], Ebenezer Chika Ikechukwu[2], Dorcas Delia Johnson[3], Zainab Lamido Tanko[4], Majekano Isa[5], Ibrahim Abdulaziz Isoga[6], Zainab Rabilu Daninna[7], Taimako Iliyasu[8], Ahmad Umar[1]

[1]Ahmadu Bello University, Zaria, Nigeria, [2]Malaria Consortium, Abuja, Nigeria, [3]Nigerian Center for Disease Control and Prevention - National Reference Laboratory, Abuja, Nigeria, [4]Kaduna State University, Kaduna, Nigeria, [5]Primary Health Care Development Agency, Adamawa, Nigeria, [6]AIDS Healthcare Foundation (AHF) Nigeria, Kogi, Nigeria, [7]Bayero University Kano, Kano, Nigeria, [8]Chupez New Dawn Limited, Abuja, Nigeria

Nigeria has the highest global malaria burden despite having control strategies historically prioritising children under five years of age and pregnant women. Heterogeneous transmission and expanding intervention coverage in some settings may alter patterns of immunity acquisition and shift malaria burden toward older age groups. This study examined temporal trends and shifts in the age profile of malaria burden across Nigeria. A retrospective longitudinal analysis of routine malaria surveillance data from Nigeria's National Health Management Information System was conducted for 2016-2025. Annual malaria episodes were adjusted following World Health Organization guidance for reporting completeness, diagnostic testing, and care-seeking behaviour. Incidence trends relative to 2016 were estimated using negative binomial regression to obtain incidence rate ratios (IRRs) with 95% confidence intervals (CIs). Changes in the proportional contribution of demographic groups to total malaria burden were assessed using fractional logit models, reporting odds ratios (ORs) with CIs. Analyses were stratified by demographic group (children under five years [U5], individuals aged five years and above [A5], and pregnant women [PW]). Among U5, uncomplicated malaria [UM] doubled (IRR 2.1, CI 1.9-2.3) and severe malaria [SM] increased nearly threefold (IRR 2.7, CI 2.3-3). Comparable increases were observed in A5 for UM (IRR 2.1, CI 1.9-2.3) and SM (IRR 2.8, CI 2.5-3.1). For PW, UM increased modestly (IRR 1.2, CI 1.1-1.4), while SM declined (IRR 0.6, CI 0.4-0.8). Fractional logit models showed a steady decline in the proportion of UM in U5 children from 2021 to 2025 (OR 0.7, CI 0.6-0.7), with a similar decline for SM (OR 0.7, CI 0.5-0.8). The proportion of A5 increased for UM (OR 1.1, CI 1.1-1.2) and modestly for SM (OR 1.1, CI 1-1.3). PW exhibited the largest proportional increases, with UM and SM odds almost fivefold (OR 4.7, CI 2.9-7.7) by 2025. Findings suggest a gradual shift of malaria burden toward older age groups in Nigeria. The recent introduction of a malaria vaccine in Nigeria may require a rethink of the target age group to maximise its efficiency.

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FLOODING EXPOSURE AND PEDIATRIC MALARIA HOSPITALIZATION IN WESTERN UGANDA

Ashley Wade[1], Annika Gunderson[2], Huipeng Liao[2], Raphael Mbusa[3], Abel Bwambale[3], Dana Giandomenico[2], Emma Baguma[3], Bonnie Shook-Sa[2], Moses Ntaro[3], Edgar Mulogo[3], Ross M. Boyce[2]

[1]Icahn School of Medicine at Mount Sinai, New York, NY, United States, [2]University of North Carolina at Chapel Hill, Chapel Hill, NC, United States, [3]Mbarara University of Science and Technology, Mbarara, Uganda

Climate change is increasing the frequency and severity of flooding, which is projected to worsen malaria morbidity and mortality in sub-Saharan Africa. While flooding's effect on malaria transmission is well-documented, few studies have examined flooding's impact on driving severe malarial disease. We investigated the association between household flooding exposure and pediatric hospitalization for malaria using baseline data from an ongoing cluster randomized trial in western Uganda. Between February-May 2025, caregivers from 3,753 households across 79 villages in three flood-prone river valleys completed surveys on flooding exposure, daily-life disruptions, and malaria hospitalizations among children ≤ 12 years in the prior year. We assessed associations between flooding exposure and child malaria hospitalization using generalized estimating equations with a Poisson distribution to estimate population-average risk ratios, accounting for village-level clustering. Flooding exposure was defined as: (i) no exposure, (ii) indirect flooding exposure (no household/property flooding but reported daily life disruptions), and (iii) direct flooding exposure (household/property flooding). Of 9,323 children, 1,501 (16%) were hospitalized for malaria in the prior year. Compared to unexposed children, children with indirect flooding exposure had 49% higher risk of hospitalization (aRR 1.49, 95% CI 1.23-1.81) and direct exposure had 77% higher risk (aRR 1.77, 95% CI 1.41-2.21). Within exposed households, flooding-related disruptions to work (aRR 1.83, 95% CI 1.52-2.20) and healthcare (aRR 1.54, 95% CI 1.26-1.87) were most strongly associated with hospitalization. Disruptions to healthcare and schooling lasting more than one week were associated with further increases in risk. These findings suggest flooding may worsen malaria burden through disrupted livelihoods and healthcare access beyond increased transmission. Our results underscore the need for interventions pairing malaria prevention with strategies to restore access to work and healthcare to reduce the burden of post-flood malaria in affected communities.

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FROM CHILDREN TO ADULTS? SHIFTING AGE PATTERNS OF MALARIA IN KENYA, 2008 - 2025

Gladys Chemwor[1], Benjamin Opot[1], Dennis Juma[1], Agnes Cheruiyot[1], Redemptah Yeda[1], Risper Maisiba[1], Maurine Mwalo[1], Jackline Jumah[1], Raphael Okoth[1], Edwin Mwakio[1], Farid Salim[1], Doris Njoroge[1], Michal Ohaga[1], Robert Haynes[2], Luis Pow Sang[2], **Hoseah M. Akala**[1]

[1]Kenya Medical Research Institute/Walter Reed Army Institute of Research, Kisumu, Kenya, [2]Walter Reed Army Institute of Research - Africa (WRAIR-A), Kisumu, Kenya

From 2000, malaria control has undergone one of the largest public-health scale-up in history. Since the interventions are tailored for targeted age-groups at highest risk, measuring its impact during a period of declining burden requires accurate accounting of malaria across ages. This study used longitudinal data to assess temporal malaria trends across age groups in selected regions of Kenya. Between 2008 and 2025, a total of 4,184 individuals aged 6 months and older, presenting at outpatient departments of hospitals located in three of the five Kenya's malaria burden zones, namely, Lake endemic, highland epidemic, and coast endemic regions, with symptoms consistent with uncomplicated malaria, were enrolled into this study. Demographics and clinical information were obtained using a structured questionnaire. Further, each participant was diagnosed for malaria parasites by microscopy. Data was analyzed using R. Lake Endemic region enrolled 2,737(65.42%), highland epidemic 1,278(30.54%) and coast

endemic 169(4.04%). The number enrolled by age categories in years; 0-5yrs 51.58% (2,158), 6-11yrs 18.79% (786), 12-17yrs 7.36% (308), 18-26yrs 13.05% (546), and 27-78yrs 9.23% (388) varied significantly, x^2 p=0.000. The median age increased significantly from 2008 (4yrs), 2013 (5yrs), 2019 (7yrs), to 2023 (13yrs), suggesting a shift in the disease burden toward older age groups, x^2 p=0.0001. The median parasite density among individuals presenting for healthcare declined significantly from 37,500/ul (n=196) in 2008 to 22,470/ul (n=398) in 2013, to 4,840/ul (n=144) in 2018, reaching 5,080/ul (n=102) in 2023, KW p=0.0001. 2024 and 2025 analysis is ongoing. The age of individuals with clinical malaria rose significantly between 2008 and 2023. In reciprocity, the parasite density decreased significantly during the same period. While these changes tend to imply ease of acquisition of infection by the less targeted age groups, which suggests less immunity or greater access to healthcare; there is a need to reassess age-targeted control to ensure that those at the highest risk of developing clinical disease are sufficiently protected.

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THE RELATIONSHIP BETWEEN PLACENTAL MALARIA AND PRE-ECLAMPSIA: A SYSTEMATIC REVIEW AND META-ANALYSIS

Dorcas Quaye[1], Peter Appiah Offori[1], Elizabeth Kate Wagman[2], Nicolette Pirjanian[3], Samuel Oppong[1], Sarah E. Boudova[3]

[1]University of Ghana, Accra, Ghana, [2]Philadelphia, PA, United States, [3]University of Illinois Chicago, Chicago, IL, United States

Preeclampsia (PE) is a placenta-mediated hypertensive disorder of pregnancy that causes significant morbidity and mortality globally, with a disproportionate burden in malaria-endemic regions. Placental malaria (PM) has an overlapping pathophysiology and may predispose women to develop PE. Observational studies have not consistently demonstrated an association between PM and PE, although they have been limited by sample size and heterogeneous study designs. We aimed to determine the association between PM and PE through a systematic review and meta-analysis, and to identify critical effect modifiers. We searched PubMed, Web of Science, and Scopus (inception to December 2025) for observational studies reporting rates of PM and hypertensive disorders of pregnancy. Random-effects meta-analyses were performed. Pre-specified subgroup analyses were conducted. Heterogeneity was quantified using I^2 and τ^2. The study protocol was registered with PROSPERO: CRD420251176614. In total, nine original articles (11,073 pregnancies) were included. PM was associated with a significantly elevated risk of PE (pooled OR 2.45, 95% CI 1.36-4.40; I^2 = 73.9%, τ^2 = 0.532). The effect wasn't driven by a specific timing of PM (acute, chronic or past). Histopathologically diagnosed PM showed a robust effect (OR 4.09, 95% CI 2.46-6.79; I^2 = 18.2%), whereas composite diagnostic approaches (histopathology plus PCR or blood smear) yielded attenuated or null effects. No significant associations were observed between PM and gestational hypertension (OR 1.73, 95% CI 0.68-4.41) or eclampsia (OR 1.00, 95% CI 0.28-3.52). This systematic review and meta-analysis showed a strong association between PM and PE, which has significant ramification for public health policy. Interventions targeting PM may have the dual benefits of preventing both malaria and PE-related morbidity and mortality and should be prioritized in antenatal care.

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SEROLOGICAL SIGNATURE SUGGESTS WIDESPREAD *PLASMODIUM VIVAX* CIRCULATION IN DUFFY-NULL POPULATIONS OF MADAGASCAR

Elysé Tahiriniaina Maminjanahary[1], Caitlin Bourke[2], Camille Roesch[1], Henintsoa Narindra Rakotondramanana[1], Hasinirina Elisabeth Elison[1], Reziky Tiandraza Mangahasimbola[1], Aina Harimanana[1], Theodora Mayouya-Gamana[1], Mirella Malala Randrianarisoa[1], Eliharintsoa Rajaonarimirana[1], Judickaelle Irinantenaina[1], Christopher Drakeley[3], Lova Tsikiniaina Rasoloharimanana[1], Matthieu Schoenhals[4], Stephane Pelleau[4], Rindra Vatosoa Randremanana[1], Ivo Mueller[5], Rila Ratovoson[1], Rhea Longley[5], Michael White[4], Benoit Witkowski[1]

[1]Institut Pasteur de Madagascar, Antananarivo, Madagascar, [2]Menzies School of Health Research, Darwin, Australia, [3]London Sch of Hygiene & Tropical Medicine, London, United Kingdom, [4]Institut Pasteur, Paris, France, [5]Walter and Eliza Hall Institute of Medical Research, Parkville, Australia

The human Duffy antigen (encoded by the *Ackr1* gene) is critical for *Plasmodium vivax* invasion of reticulocytes. The majority of the African population carries -67 T/C mutation in *Ackr1* leading to a Duffy-null phenotype, which explains the lower *Plasmodium vivax* detection in Africa. However, recent studies suggests that Duffy-null individuals may be infected with *P. vivax,* with parasites sequestering in receptive tissues such as the bone marrow or other extravascular compartments. These localizations evade current diagnostic approaches (RDTs, microscopy and PCR), suggesting that *P. vivax* may circulate in Africa at a much higher level than previously recognized. Therefore, *P. vivax* serology may represent a viable tool to redefine *P. vivax* epidemiology in Africa. To explore this, we analysed the *P. vivax*-specific serological responses and Duffy genotypes in Malagasy individuals: first in an asymptomatic population (n=3077) from Mandoto district where *P. vivax* actively circulates and then in individuals (n=3472) consulting local health facilities, including areas without reported *P. vivax* circulation. *P. vivax* serological signatures were assessed using a multiplexed antigen panel and associated with RT-PCR *P. vivax* detection. Duffy genotype was determined using a TaqMan probe-based assay. In Mandoto, using PCR and serology, the prevalence of *P. vivax* in the Duffy-positive population was 16% and 52.8%, respectively (3-fold higher serological detection). In the Duffy-null population, prevalence was 1.7% and 24.8%, respectively (15-fold higher). Madagascar-wide samples showed similar trends, with 24.8% vs. 1.5% PCR positivity in Duffy-positive and -null, respectively, while serology showed 35.1% vs. 20.7%. *P. vivax* seropositivity, indicative of recent *P. vivax* exposure, suggests widespread presence of *P. vivax* in all populations. In line with emerging literature, this study confirms that Duffy-null individuals are not fully protected from *P. vivax* and that current tools detecting only peripheral circulating parasites are inadequate for this population. *P. vivax* circulation in Madagascar and Africa may be considerably underestimated.

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BEYOND TEMPERATURE: HUMIDITY AS A MISSING DRIVER OF URBAN MALARIA RISK

Courtney Murdock[1], Paul Huxley[2], Brandyce Laurent[1], Britny Leigh Johnson[3], Brandon D. Hollingsworth[4], Leah R. Johnson[5], Michael C. Wimberly[6], Joel Brown[1], Mercedes Pascual[7], Sachin Sharma[8]

[1]Cornell University, Ithaca, NY, United States, [2]University of York, York, United Kingdom, [3]Ithaca, NY, United States, [4]Cornell University, Columbia, SC, United States, [5]Virginia Tech, Blacksburg, VA, United States, [6]University of Oklahoma, Norman, OK, United States, [7]New York, NY, United States, [8]Cornell University in India, Delhi, India

Urban environments are among the fastest growing landscapes globally, accompanied by marked increases in vector-borne disease transmission by urban-adapted mosquitoes. One key vector, *Anopheles stephensi*, drives urban malaria transmission across South Asia and poses an emerging threat to Africa. While temperature is known to exert strong, nonlinear effects on malaria transmission, the role of relative humidity

remains understudied and is often modeled separately from temperature despite likely interactions. This gap is particularly important for container-breeding urban mosquitoes, for which precipitation is a weak predictor of transmission because water storage and artificial containers can persist as larval habitats without rainfall. To address this critical gap, we conducted high resolution, full factorial environmental experiments that investigated the response of juvenile traits (probability of juvenile survival and juvenile development rate), adult traits (lifespan, daily egg production, and daily biting), and malaria traits (*Plasmodium falciparum* vector competence and parasite development for 24 days post-infection) across a range of constant temperatures (12-40 C) and relative humidities (30%-90%). We used Bayesian hierarchical models to investigate the effects of these treatments on mosquito and parasite traits relevant for transmission. From these analyses, we integrated environment-trait relationships into a relative model for the basic reproductive number of malaria (relative R_0) to estimate *P. falciparum* environmental suitability. We show that relative humidity variation dramatically alters predicted thermal suitability for malaria transmission, resulting in distinct spatial and seasonal structure for climatic suitability in both the native (S. Asia) and invasive zones (Africa). These findings challenge temperature-only projections and highlight moisture availability as an important, overlooked axis of climatic suitability for disease vectors and disease transmission.

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DETERMINANTS OF CLONE-LEVEL PERSISTENCE IN SUBCLINICAL *PLASMODIUM FALCIPARUM* INFECTIONS: A LONGITUDINAL COHORT STUDY IN WESTERN KENYA

Elizabeth Hemming-Schroeder[1], Brook Jeang[2], Lauren Bradley[3], Ming-Chieh Lee[4], Daibin Zhong[5], Arlene Dent[6], James W. Kazura[7], Harrysone Atieli[8], Guiyun Yan[4]

[1]Colorado State University, Fort Collins, CO, United States, [2]Walnut, CA, United States, [3]Irvine, CA, United States, [4]University of California, Irvine, Irvine, CA, United States, [5]Univ of California Irvine, Irvine, CA, United States, [6]Medpace, Cincinnati, OH, United States, [7]Case Western Reserve University, Cleveland, OH, United States, [8]Maseno Univ, Kisumu, Kenya

Individuals with persistent, subclinical *Plasmodium falciparum* infections contribute disproportionately to onward transmission and may sustain transmission during low-vector periods, yet the factors governing infection persistence remain poorly understood. We characterized persistence of subclinical infections using monthly dried blood spot samples collected in Homa Bay, Kenya (2019-2021). Parasite strains were resolved using the Pfcpmp marker, enabling longitudinal identification of unique clones within individuals over time. We additionally quantified parasite density and measured host antibody reactivity to multiple *P. falciparum* antigens. Time-to-event models were used to evaluate predictors of clone persistence. We identified several significant relationships. Higher reactivity to PfMSP2 was associated with greater infection complexity ($p = 0.029$), while higher PfCSP reactivity was associated with lower parasite density ($p < 0.001$). However, neither infection complexity nor parasite density predicted persistence. Instead, timing within season was the strongest predictor: clones were more likely to be cleared in the early rainy season and least likely to be cleared in the late rainy and early dry seasons ($p = 0.021$). Age and sex were not significant predictors of persistence. These findings suggest that while host immunity shapes infection complexity and parasite density, it does not fully explain persistence dynamics. Rather, these findings support the hypothesis that seasonality is a dominant driver of persistence, potentially reflecting parasite-intrinsic shifts in transcriptional state. Parasites may adopt persistence-associated phenotypes that enable long-term survival and reduced susceptibility to clearance.

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IMPORTED PEDIATRIC MALARIA IN THE UNITED STATES: IDENTIFYING RISK FACTORS AND AREAS OF NEED

Sesh Alexander Sundararaman[1], Brianne Roper[2], Amy K F Davis[3], Karen L. Hanze Villavicencio[4], Jonathan Mayhew[5], Michelle L. Wang[6], Nina Lei Tang[7], Vijaya L. Soma[8], Ziyi Wang[1], Margaret Feeney[9], Indi Trehan[10], Jill Weatherhead[11], Chandy C. John[12], Jeffrey S. Gerber[1], Audrey Ragan Odom John[13]

[1]The Children's Hospital of Philadelphia, Philadelphia, PA, United States, [2]University of Pennsylvania, Philadelphia, PA, United States, [3]University of California San Francisco, San Francisco, CA, United States, [4]Children's Hospital of Philadelphia, Philadelphia, PA, United States, [5]Western Michigan University School of Medicine, Kalamazoo, MI, United States, [6]University of Washington School of Medicine, Seattle, WA, United States, [7]Houston, TX, United States, [8]NYU Grossman School of Medicine, New York, NY, United States, [9]Univ of California, San Francisco, San Francisco, CA, United States, [10]University of Washington, Lake Forest Park, WA, United States, [11]National School of Tropical Medicine Baylor College of Medicine, Houston, TX, United States, [12]Indiana University School of Medicine, Saint Paul, MN, United States, [13]Children's Hospital of Philadelphia, Swarthmore, PA, United States

Although malaria is no longer endemic to the United States, approximately 2,000 cases of imported malaria are diagnosed in the country each year, 10-20% of which occur in children. Pediatric travelers represent a distinct epidemiologic group with different reasons for travel and risks for infection or severe outcomes compared to adults. In this study, we characterize imported pediatric malaria in the United States, examining patient demographics, clinical outcomes, and risk factors for severe disease. We conducted a retrospective descriptive study of pediatric malaria cases at nine hospitals in the U.S. between 2016 and 2023. Cases were identified using ICD-10 codes and data extracted via chart review. We identified 171 children treated for malaria. The majority of patients had traveled to West Africa, and 60% reported traveling to visit friends or relatives. One-third of patients received antimalarial prophylaxis, but adherence to prophylactic regimens was poor. Thirty-two percent of patients were diagnosed with severe malaria. In multivariate analysis, we found that delays in the diagnosis of malaria at initial presentation in the U.S. were associated with an increased risk of severe disease (adjusted odds ratio [aOR], 4.8). Using health insurance claims data, we also examined costs associated with in-hospital malaria treatment. This analysis shows that the costs associated with treatment of severe malaria have increased significantly over the last five years, likely reflecting the costs of IV artesunate in the U.S. healthcare system. Our findings highlight the need for improved malaria prevention for U.S. pediatric travelers - especially those visiting friends and relatives - as well as the importance of prompt recognition and testing of returned travelers at risk of malaria.

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CROSS-SECTIONAL ANALYSIS OF A CHAGAS CARDIOMYOPATHY COHORT IN BOLIVIA: EPIDEMIOLOGIC AND CLINICAL DETERMINANTS IN AN URBAN HOSPITAL-BASED POPULATION

Brandon N Mercado-Saavedra[1], Shilah Waters[2], Marcelo Buhezo[3], Manuela Verastegui[4], Edith Malaga[5], Adriana Hernani[6], Paula Carballo[6], Gustavo Duran[6], Freddy Tinajeros[6], Robert H. Gilman[7]

[1]Instituto de Investigación de Medicina, Universidad Catolica Boliviana San Pablo, Santa Cruz, Plurinational State of Bolivia, [2]Department of International Health Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [3]Asociación Benefica PRISMA, Santa Cruz, Plurinational State of Bolivia, [4]Laboratorio de Investigación y Desarrollo, Universidad Peruana Cayetano Heredia, Lima, Peru, [5]Laboratorio de Investigación y Desarrollo, Universidad Cayetano Heredia, Lima, Peru,

[6]Asociacion Benefica PRISMA, Santa Cruz, Plurinational State of Bolivia, [7]Bloomberg School of Public Health, Johns Hopkins Univers, Baltimore, MD, United States

Chagas disease is a neglected tropical disease primarily affecting Latin America, with increasing spread to non-endemic countries through migration and vertical transmission. A major consequence is Chagas cardiomyopathy (CC), a non-reversible condition affecting approximately one-third of infected individuals, with substantial clinical and economic impact. Bolivia has the highest global prevalence, and although transmission historically occurred in rural areas, urban hospitals are now seeing increasing numbers of CC cases. We describe one of the largest CC cohorts, including 4,112 adults recruited at a hospital in Santa Cruz, Bolivia (2014-2022). Participants underwent serologic testing, interviews, ECG, and echocardiography. Cardiomyopathy severity was classified according to the modified Acquatella criteria. Multivariable logistic regression models identified factors associated with *Trypanosoma cruzi* seropositivity, accounting for familial clustering using generalized estimating equations. Overall, 57.4% were seropositive. Seropositive individuals were older, more often male, had lower education, and greater exposure to rural environments and poor housing, including adobe walls, palm roofs, and triatomine infestation. In adjusted models, seropositivity was associated with age (OR≈1.05/year), male sex (OR≈1.30), family history (OR≈3.4), and a composite housing index (OR≈1.29). Longer triatomine exposure increased the odds of infection, while higher education was protective. Higher heart rate and pulse pressure were inversely associated with seropositivity. Disease severity increased with age and was associated with male sex, lower education, rural exposure, prolonged vector exposure, and poor housing. Advanced stages showed a higher prevalence of cardiovascular symptoms, arrhythmias, and heart failure. In this large urban cohort, *T. cruzi* infection and cardiomyopathy severity reflect cumulative social and environmental exposures, highlighting the lasting impact of rural risk factors and the need for prevention strategies targeting housing and structural determinants.

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VALIDATION OF RPA (RECOMBINANT POLYMERASE AMPLIFICATION WITH LATERAL FLOW) AS A DIAGNOSTIC TEST FOR CONGENITAL CHAGAS IN HEALTH CENTERS, BOLIVIA, 2026

Emily Joany Arteaga carrasco[1], Brenda Vasquez[2], Freddy Tinajeros[3], Robert H. Gilman[4], Natalie M. Bowman[5]

[1]Emily, Andres Ibañez, Plurinational State of Bolivia, [2]University of North Carolina Chape Hill School Of Public Health, Andres Ibañez, NC, United States, [3]Johns Hopkins Univ, Santa Cruz, Plurinational State of Bolivia, [4]Johns Hopkins Univ Bloomberg School of Public Health, Baltimore, MD, United States, [5]Univ of North Carolina, Chapel Hill, NC, United States

Vertical transmission represents a growing proportion of new Chagas cases in Bolivia, with a transmission rate of 6% (Klein, 2020). Bolivia lacks standardized testing to diagnose Congenital Chagas in newborns in a timely manner, leading to treatment delays. RPA-LF (Recombinant Polymerase Amplification combined with Lateral Flow) is a method that could be useful for diagnosing Congenital Chagas disease at a low cost in Bolivian health centers, allowing for timely treatment and reducing the disease's prevalence in the population. The objective of this study is to validate the RPA-LF test as a diagnostic tool for Congenital Chagas in health centers across Bolivia. Therefore, this study seeks to improve diagnosis through cost-effective, sensitive, and specific techniques.A sample of 413 children born in the maternity hospital was analyzed, with mothers providing consent for study inclusion. Samples included umbilical cord blood, peripheral blood, and follow-up samples at 1 month and 9 months. An evaluation of the test's sensitivity and specificity was performed against the gold standard (qPCR) using Epidat 3.1. The evaluation results showed: Sensitivity: 95% (95% CI: 82.95–100.00); the test is capable of detecting 95% of newborns who have the condition. Specificity: 100% (95% CI: 99.87–100.00); indicating it is ideal for identifying healthy subjects.Youden's Index: 0.95 (indicative of excellent test performance).PPV (Positive Predictive Value): 100.00% NPV (Negative Predictive Value): 99.74%The evaluated test demonstrates excellent diagnostic performance. Its strongest point is the 100% specificity, making it an ideal tool for confirming a diagnosis. Although there is a small margin of error in sensitivity (1 false negative), its overall ability to discriminate between healthy and infected individuals in this sample is very high. It represents a viable option for diagnosing Congenital Chagas in health centers with limited equipment.

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TOCSINS: A PHASE 2 ADAPTIVE PLATFORM TRIAL OF COMMUNITY-ADMINISTERED ORAL SNAKE VENOM METALLOPROTEINASE (SVMP) INHIBITORS FOR SNAKEBITE ENVENOMING IN BRAZIL AND GHANA

Michael Abouyannis[1], Zoe Johnston[1], Laura-Oana Albulescu[1], Tansy Edwards[1], Tom Fletcher[1], Richard FitzGerald[2], Becky Jones-Phillips[1], Benno Kreuels[3], Mainga Hamaluba[4], Charlotte Rawcliffe[1], Timothy F. Platts-Mills[5], Marco Aurélio Sartim[6], Ymkje Stienstra[1], Jeroen Kool[7], Sofia Moco[7], Saye H. Khoo[2], David G. Lalloo[1], Wuelton Marcelo Monteiro[6], John A. Amuasi[8], Nicholas R. Casewell[1]

[1]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [2]University of Liverpool, Liverpool, United Kingdom, [3]Bernhard-Nocht Institute for Tropical Medicine, Hamburg, Germany, [4]KEMRI-Wellcome Trust Research Programme, Kilifi, Kenya, [5]Ophirex, Corte Madera, CA, United States, [6]Fundação de Medicina Tropical Dr. Heitor Vieira Dourado, Manaus, Brazil, [7]Vrije Universiteit, Amsterdam, Netherlands, [8]Kumasi Centre for Collaborative Research in Tropical Medicine, Kumasi, Ghana

Snakebite envenoming, a WHO-recognised neglected tropical disease, causes an estimated 1.8 million envenomings and 94,000 deaths annually. Venom-induced consumption coagulopathy (VICC), driven by snake venom metalloproteinases (SVMPs) across many medically important snake species, is a major contributor to morbidity and mortality worldwide. Antivenom is the only approved treatment but is limited by high cost, risk of anaphylactoid reactions, cold-chain requirements, and the need for intravenous administration in hospital settings, restricting timely access. Orally administered SVMP inhibitors (SVMPi), including repurposed drugs with established human safety data, may enable earlier, decentralised treatment. Lead candidates include 2,3-dimercapto-1-propanesulfonic acid (DMPS), a metal chelator, and marimastat, a hydroxamate-based matrix metalloproteinase inhibitor; both have favourable safety profiles in humans and broad preclinical efficacy against envenoming from diverse snake species. The TOCSINS trial is a multicentre, multi-arm, phase 2 Bayesian adaptive platform trial in Brazil and Ghana evaluating oral SVMPi for snakebite. Adults presenting within 12 hours of snakebite are enrolled across two stages. In Stage A, participants with VICC (INR ≥1.4) are randomised in community settings to receive oral SVMPi or placebo prior to referral, with all participants receiving antivenom on hospital arrival; this stage assesses for a proof-of-concept efficacy signal. Bayesian analyses assess time to coagulopathy resolution. In Stage B, treatment arms demonstrating efficacy in Stage A are compared against intravenous antivenom in hospital settings, to evaluate their potential to replace antivenom for SVMP-predominant species. This two-stage design enables a cautious, stepwise transition away from antivenom while maintaining safety. This adaptive platform allows efficient evaluation of multiple candidates.

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TOWARD OPTIMIZING STRONGYLOIDIASIS TESTING IN AT-RISK U.S. PATIENTS: COHORT CHARACTERISTICS AND EARLY LONGITUDINAL FINDINGS

Cora Alperin, Duc T. Nguyen, Cameron Brown, Megan Duffey, Eva H. Clark

Baylor College of Medicine, Houston, TX, United States

Strongyloidiasis, with an estimated global burden of 30–100 million cases, is a common, potentially life-threatening neglected tropical disease that remains underdiagnosed due to low provider awareness and imperfect diagnostics. The clinical diagnostic standard, evaluation of multiple

stool specimens by ova-and-parasite microscopy, has poor sensitivity; consequently, serologic testing is widely used despite suboptimal performance. The commonly used, *Strongyloides* crude antigen IgG ELISA (IgG), takes >1 year to serorevert. We are conducting a clinical laboratory–based study collecting paired serum and stool samples to evaluate the performance of a panel of *Strongyloides* diagnostic tests. Positive patients are treated and followed with serial testing for two years to evaluate panel components as tests-of-cure. Here we describe the preliminary cohort. Among 180 adults with available IgG results, median age is 50 years (interquartile range [IQR] 42, 58); 56% are male, 72% Hispanic; 33% U.S.-born and 33% born in Mexico. Immunosuppressive conditions are common, including HIV (27.8%, median CD4 count 34.5 cells/mm^3, IQR 16, 186), diabetes (58.2%; median HbA1c 6.8%, IQR 6.0, 8.4), malignancy (28.2%), and iatrogenic immunosuppression (14.6%). Overall *Strongyloides* IgG seropositivity is 22.8% (41/180). Of 68/180 patients who met criteria for significant immunosuppression, defined as HIV with CD4 <500; diabetes with HbA1c ≥8%; malignancy with ANC <500; post–solid organ transplant; or receipt of immunosuppressive medication, 37% were seropositive. Among 20 seropositive patients with follow-up data, 15.3% seroreverted by 3 months after treatment and 32.2% by 6 months. IgG tended to decline (–0.32 (95% CI –0.52, 0.18), p=0.21). Strongyloidiasis exposure is substantial in this high-risk U.S. cohort. Although most patients maintain prolonged seropositivity, a subset demonstrate rapid serologic clearance, which may reflect heterogeneity in infection burden, timing of exposure, or host and treatment factors. Specimen collection, patient enrollment and follow-up, stool PCR, and serum ELISA testing are ongoing.

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LEISHMANIASIS IN A U.S. HEALTHCARE SYSTEM SERVING AN AT-RISK POPULATION: RETROSPECTIVE EVALUATION OF EPIDEMIOLOGIC AND DIAGNOSTIC FEATURES OVER 15 YEARS

Aileen Y. Hu, Cora Alperin, Eva Clark

Baylor College of Medicine, Houston, TX, United States

Leishmaniasis is a neglected tropical disease with an estimated 700,000-1 million new cases annually. Caused by *Leishmania* protozoa transmitted by sandflies, it classically manifests as cutaneous (CL), mucocutaneous (MCL), or visceral (VL) disease. In the US, several hundred cases are diagnosed annually, primarily in migrants and travelers from endemic regions, though locally acquired CL occurs in Texas. Leishmaniasis is likely underdiagnosed in the US due to low provider awareness and limited access to specieslevel diagnostics. This study evaluated healthcare provider behaviors in a Texas health system serving a large at-risk population to identify diagnostic delays. We retrospectively identified adult patients diagnosed with leishmaniasis in Harris Health between 2010-2025 via ICD-9 or -10 code. We extracted demographic, clinical, and provider behavior variables from the electronic health record using a standardized data collection form. Of 34 patients identified, 18 truly were diagnosed with leishmaniasis (median age 39 years [IQR 16], 67.7% male). Most patients (n=14) reported residence or travel in Latin America; the remaining 4 reported residence in Afghanistan, Ethiopia, India, and Pakistan. No infections were acquired locally in Texas. Of the 18 cases, 13 were CL, 4 MCL, and 1 VL. All CL/MCL cases underwent skin tissue biopsy. 11/13 CL and 4/4 MCL cases underwent species-level PCR; 11 had species in the *L. Viannia* subgenus. Among CL cases, the median time-to-diagnosis (TTD) was 2 months (range 1-12). For MCL, median TTD was 6 months (range 2-12). MCL TTD was prolonged due to 2/4 cases being complicated by advanced HIV and disseminated disease. The single VL case—also with HIV co-infection—was diagnosed clinically; bone marrow biopsy was negative. In our health system, CL and nondisseminated MCL usually were diagnosed within a few months of symptom onset. In contrast, diagnosis of disseminated presentations was delayed. Further study of this cohort will evaluate provider management practices and interventions to improve provider awareness of guideline-based approaches to leishmaniasis diagnosis and management.

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MULTIPLEXED AND MULTIPARALLEL QPCR ASSAY FOR THE SIMULTANEOUS DETECTION OF THE MAJOR PARASITIC CAUSES OF EOSINOPHIL MENINGITIS

Joseph Kubofcik[1], William J. Sears[2], Sasisekhar Bennuru[1], Elise M. O'Connell[1], Thomas B. Nutman[1]

[1]National Institutes of Health, Bethesda, MD, United States, [2]National Institutes of Health, Silver Spring, MD, United States

Eosinophilic meningitis (EoM) is a rare and occasionally fatal form of meningitis most often caused by infection with helminth parasites. To develop a rapid, specific and ultrasensitive single assay (multiplexed or multiparallel) to detect in CSF the free DNA of the major parasitic causes of eosinophil meningitis, we used existing qPCR assays (for *Angiostrongylus cantonensis* and *Taenia solium*) and developed new highly specific qPCR assays for *Baylisascaris procyonis*, *Gnathostoma spinigerum*, *Toxocara canis*, and *Toxocara cati*, based on the Repeat Explorer algorithm using the short read archives (SRAs) of each of these parasite species. Each qPCR assay developed is 100% specific for the individual parasite species (does not cross amplify each other's parasite genomic DNA) nor do they amplify human genomic DNA. Each individual qPCR assay has a limit of detection between 10 and 1 fg of genomic DNA. To enable a clinically reliable assay, we developed oligonucleotide targets for all 6 parasite targets and developed a plate based ready-to use system that allows for the simultaneous detection of DNA from the 6 EoM-causing helminth parasites in a reproducible and clinically validated format that can provide a species-specfic diagnosis within an hour of receiving a CSF sample. In addition, by putting different fluorophores on each of the 6 qPCR assay probes, we have been able to perform these assays in a single well (i.e. multiplexed), but this approach requires more sophisticated instrumentation and greater expense. This assay has already been useful in defining the causative agent of EoM in CSF from 4 patients in whom the definitive diagnosis was unclear. The ability to detect the presence of DNA from these 6 major causes of EoM should allow for more targeted (and appropriate) therapy to be given to patients with EoM with the potential of improving outcomes.

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WHY IS POINT-OF-CARE ULTRASOUND (POCUS) SO UNDERUSED IN TROPICAL MEDICINE? - A SCOPING REVIEW

Victoria Vezzoli[1], Charles E. Bares[1], Armanda Leka[1], Gian Luca D'Alessandro[2], Ambra Raimondi[3], **Enrico M. Brunetti**[4]

[1]University of Pavia, Pavia, Italy, [2]ASST Melegnano Martesana, Milan, Italy, [3]San Matteo Hospital Foundation, Pavia, Italy, [4]University of Pavia, San Matteo Hospital Foundation, Pavia, Italy

Introduction: Point of Care Ultrasound (POCUS) is increasingly used across medical specialties, including Infectious diseases (ID), but remains underutilized in tropical medicine. We conducted a review of existing applications of POCUS relevant to an infectious disease clinician, focusing specifically on applications in tropical diseases, and identified trends over the last two decades. Methods: We conducted a scoping review following the protocol by Arksey and O'Malley of papers on POCUS in the management of infectious conditions, published from 2007 to 2025. Data recorded included year and type of publication, causative microorganism, and diagnosis where applicable. Results: A total of 168 papers were included in the analysis. The most common type of publication was a case report (n = 60) discussing or highlighting the utility of POCUS in a single case. Since 2007, there has been a massive increase in the number of yearly publications, especially case reports (n = 1 to n = 13). The most frequent application across all paper types was investigation of respiratory conditions (n = 45) followed by septic arthritis (n = 33) and HIV (n = 15). However, only a small fraction of these papers is related to tropical diseases (n = 13) and even when expanded to include infections that are overwhelmingly prevalent in tropical countries such as tuberculosis, tropical disease still represents a small minority of publications (n = 46). Discussion: POCUS for infectious diseases has a broad range of clinical applications.

Paradoxically, while the potential for tropical diseases is even broader, given the lack of advanced imaging such as computed tomography in tropical areas and the complexity of the conditions, this is where POCUS is least used. We discuss ways to improve the situation.

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LABORATORY DIAGNOSIS OF MYCETOMA IN SENEGAL: A MULTIDISCIPLINARY APPROACH THROUGH SATELLITE LAB HUBS AND MOLECULAR REFERENCE LAB

Doudou Sow[1], Maguette Faye[1], Pape Ndiole Diagne[1], Carole Pab Minlekib[1], Wendy WJ Van De Sande[2]

[1]Department of Parasitology-Mycology/UGB, Saint-Louis, Senegal, [2]ErasmusMC, Gilze, Netherlands

Mycetoma is a chronic disease that is caused either by bacteria or fungi producing grains. The diagnosis of species is important to guide the therapeutic management of patients. However, the identification of the causative agents is challenging in our resources limited settings. This study aims to assess the implementation of molecular techniques versus the mycological and histological methods. A prospective study was carried out from January 2022 to August 2023. Capacity building of peripheral satellite labs have been done for the confirmation of cases. In addition, a molecular reference lab has been set up in the north of Senegal. All the suspected mycetoma cases in three hospitals have been screened using mycological, histological and molecular techniques. Biopsy specimens and/or grains obtained from patients were examined by PCR targeting the ITS (fungal agents) and the HSP65 gene (for *Actinomadura* and *Streptomyces)*. Sequencing was performed to identify the species. A total of 67 patients have been enrolled. The mean of age was 38 years. There was more male (68.6%) than female (31.4%). There was 34.3% of black grains, 28.3% of white grains and 14.9% of red grains. The molecular techniques have identified for the first time in Senegal *Madurella tropicana* and *Gordonia sp*. The other species including *Madurella mycetomatis* and *Actinomadura pelletieri* have been identified using microbiological methods confirmed by ITS and 16S PCR followed by sequencing. National and international trainings have been done to improve the capacity of the molecular reference lab. These results show the importance of molecular techniques to improve the diagnosis of mycetoma. The implementation of these techniques will help the ministry of health for the control of mycetoma.

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NEUROLOGIC SEQUELAE IN LIBERIAN PEDIATRIC EBOLA VIRUS DISEASE SURVIVORS 10 YEARS AFTER INFECTION

Hanalise V. Huff[1], John Tweh[2], Princess Lobbo[3], Victor Taryor[3], Joseph Dorbor[3], Rebecca Aba Slewion[3], Gina Norato[1], Leroy Yankae[4], Avindra Nath[1], Dehkontee Dennis[3], Kumblytee Johnson[3], B. Jeanne Billioux[1]

[1]National Institutes of Health, Bethesda, MD, United States, [2]Prevail, Monrovia, Liberia, [3]PREVAIL, Monrovia, Liberia, [4]JFK Medical Center, Monrovia, Liberia

The 2014 West Africa EVD epidemic infected over 28,000 people, mostly in the countries of Liberia, Guinea, and Sierra Leone. The long-term impact of EVD on the developing brain was largely unknown. This study aimed to characterize the neurologic sequelae following Ebola Virus Disease (EVD) in Liberian children ten years following infection. This cross-sectional observational study enrolled EVD survivors (cases) and their close contacts (controls) in Monrovia, Liberia between February and September 2025. A single visit included neurologic symptom questionnaire, neurologic exam, and cognitive testing via NIH Toolbox Cognition Battery (NIHTB-CB) and International Cognitive Assessment-Pediatrics (ICA-P). Symptom prevalence and neurologic exam findings were compared using Fisher's exact test. Comparisons adjusting for age, sex and education for test scores used multiple linear regression. One-hundred fifty-three cases and 175 controls were seen with median age 20 years (range 9-27) and 55% female. The prevalence of memory problems ($p<0.001$), confusion ($p=0.0012$), and vision problems ($p=0.0288$) were significantly greater in cases than controls. On NIHTB-CB, attention was significantly worse for cases than controls ($p=0.01$) with no significant difference in other cognitive domains. Scores did not differ significantly between groups on ICA-P ($p=0.468$). Neurologic exam findings were significantly worse in survivors for affect ($p=0.0384$), receptive language ($p=0.0064$), facial strength ($p=0.0149$), tongue movement ($p=0.0475$), tone ($p=0.0209$), tremor ($p=0.0005$), right lower extremity reflex ($p=0.0367$), and coordination ($p=0.0139$). On Modified Rankin Scale, survivors reported significantly more disability than controls ($p<0.0001$). On executive function assessment, survivors had significantly more trouble completing tasks independently ($p=0.005434$). On Patient Reported Outcome Measurement Information System (PROMIS) cognition questionnaire, survivors were found to have significantly worse scores ($p<0.0001$), however there were no differences between groups on PROMIS fatigue and sleep.

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CLINICAL MANIFESTATIONS AND ASSOCIATED FACTORS WITH COMPLICATED DENGUE IN OLDER PATIENTS: A PROSPECTIVE MULTICENTER STUDY IN VIETNAM

Thang Van Dao[1], Binh Nhu Do[2], Minh Duc Pham[3], Tung Huu Ha[4], Hoang Cong Nguyen[5], Vinh Hai Vu[6], Phuoc Ba Nguyen[7], Kien Trung Nguyen[8], Tuyen Van Duong[9]

[1]Department of Infectious Diseases, Military Hospital 103, Vietnam Military Medical University, Ha Noi, Vietnam, [2]Department of Military Science, Military Hospital 103, Vietnam Military Medical University, Ha Noi, Vietnam, [3]Department of Nutrition, Military Hospital 103, Vietnam Military Medical University, Ha Noi, Vietnam, [4]Director Office, General Hospital of Agriculture, Ha Noi, Vietnam, [5]Director Office, Thai Nguyen National Hospital, Thai Nguyen, Vietnam, [6]Infectious and Tropical Diseases Department, Viet Tiep Hospital, Hai Phong, Vietnam, [7]Director Office, Kien An Hospital, Hai Phong, Vietnam, [8]Department of Health Promotion, Faculty of Social and Behavioral Sciences, Hanoi University of Public Health, Ha Noi, Vietnam, [9]School of Nutrition and Health Sciences, Taipei Medical University, Taipei, Taiwan

Older adults with dengue experience a greater burden of adverse outcomes than younger patients. However, the evidence base in low- and middle-income countries (LMICs), especially in Vietnam, remains limited in the elderly. This study aimed to investigate clinical characteristics of older adults with dengue and identify associated factors with complicated outcomes in this group. This prospective observational cohort study was conducted at five hospitals in Vietnam from September 2024 to September 2025. Hospitalized dengue adults (age ≥18 years) without severe dengue at admission were included for analysis. Demographic and clinical symptoms were collected through a questionnaire, while laboratory parameters were obtained from medical records. Complicated outcomes were determined on the day of discharge. We used multivariate logistic regression to examine factors associated with complicated dengue in older individuals (age ≥60). Of the 779 cases, 207 (26.6%) were elderly. The older patients suffered more complicated disease (56.5% vs. 40.2%) and stayed in hospital longer (mean 8.4 vs. 6.9 days) ($p<0.001$). The elderly were more likely to be female, have diabetes, congestive heart failure, and chronic kidney disease. They also more frequently presented with nausea and warning signs, including vomiting, abdominal pain, and restlessness. They had higher values in white blood cells, neutrophils, glucose, aspartate aminotransaminase (AST), and urea, while having lower values of red blood cells, sodium, and chloride. Multivariate logistic results indicated that dizziness was associated with decreased likelihood of complicated dengue, whereas increased levels of AST, alanine aminotransaminase (ALT), and decreased values of platelets and sodium were associated with increased likelihood of complicated dengue in the elderly. Older adults were at higher risk of complicated dengue and prolonged length of stay. Dizziness, platelets, liver enzymes, and sodium were associated with complicated dengue in this high-risk population.

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ASSESSING RECOMMENDATIONS FOR DENGUE VACCINE TAK-003: VACCINE GUIDANCE TASK FORCE OF THE INTERNATIONAL SOCIETY OF TRAVEL MEDICINE

Lin H. Chen[1], Helena H. Askling[2], Sarah L. McGuinness[3], Dipti Patel[4], Camilla Rothe[5], Steve Schofield[6], Robert Steffen[7], Leo Visser[8]

[1]Mount Auburn Hospital, Cambridge, MA, United States, [2]Karolinska Institute, Stockholm, Sweden, [3]Monash University, Richmond, Australia, [4]National Travel Health Centre and Network (NaTHNaC), London, United Kingdom, [5]Ludwig Maximillian University Munich, Munich, Germany, [6]Department of National Defence, Canada, Dunrobin, Canada, [7]Univ of Zurich Travel Clinic, Zurich, Switzerland, [8]Leiden University Medical Center, Leiden, Netherlands

Disruptions to the US Advisory Committee on Immunization Practices (ACIP) starting in 2025 have raised concerns about the trustworthiness of newly-issued US guidance. The dismantling impacted clinicians practicing in the US as well as many countries that have referred to ACIP guidance. Consequently, clinicians may consult vaccine guidance from multiple countries when advising travelers. The ISTM appointed a task force of experts from countries with strong national immunization advisory groups to share insights. The dengue vaccine TAK-003 is now licensed in >40 countries to prevent this common travel-related infection, and travelers seek advice on TAK-003 vaccination despite lack of licensure in countries including Canada/Japan/USA. This project describes and compares national guidance for TAK-003 dengue vaccine. The Task Force emailed advisory committee representatives from countries that have high-volume outbound travelers visiting dengue-endemic destinations, to complete a structured survey capturing TAK-003 licensing status, advisory body characteristics, serostatus-specific recommendations, guidance for vaccine use, evidence review processes, and vaccine access and pricing. Respondents provided recommendation wording and relevant URLs. Data were collated and analyzed descriptively. Most surveyed European countries had licensed TAK-003 and issued national recommendations; Australia's review was ongoing; Canada/US have not licensed TAK-003. Advisory bodies consistently recommended TAK-003 for travelers with laboratory-confirmed prior dengue, but diverged on seronegative/unknown serostatus. Retrospective serology was generally not advised. Although EMA licensed for age 4 years and up and WHO recommends for dengue-naïve in endemic areas, heterogeneity exists in approach to age, dengue-naïve travelers and evidence for prior dengue infection among national TAK-003 guidance. Understanding these similarities and differences supports clearer, more consistent travel medicine counselling especially when travelers encounter conflicting online information.

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LEPTOSPIROSIS SEROSURVEILLANCE DURING MILITARY TRAVEL

Dawn L. Quigley[1], Le Jiang[2], Robyn A. Stoddard[3], Claire Kuo[4], Derek Larson[1], David Tribble[5], Tahaniyat Lalani[4]

[1]Naval Medical Readiness Training Command San Diego, San Diego, CA, United States, [2]Naval Medical Research Command, Silver Spring, MD, United States, [3]CDC, Atlanta, GA, United States, [4]Infectious Disease Clinical Research Program, Department of Preventive Medicine and Biostatistics, Uniformed Services University of the Health Sciences; Henry M Jackson Foundation for the Advancement of Military Medicine, Inc., Bethesda, MD, United States, [5]Infectious Disease Clinical Research Program, Department of Preventive Medicine and Biostatistics, Uniformed Services University of the Health Sciences, Bethesda, MD, United States

Leptospirosis, a globally distributed zoonosis, has potential military operational impact; yet, populationbased data among U.S. active duty servicemembers (ADSM) traveling outside the continental U.S. (OCONUS) remain limited. We evaluated seroprevalence and seroincidence of leptospirosis in TravMil, a prospective cohort of ADSM traveling OCONUS for ≤6.5 months between 2010-2019. ADSM enrolled pre- or post-travel; those who completed a posttravel survey, reported freshwater and/or rodent exposure, and provided a posttravel serum specimen or paired pre/posttravel sera were included. Posttravel sera were screened at 1:100 dilution by ELISA; corresponding pre-travel sera of positive samples were tested. Confirmatory microscopic agglutination testing (MAT) was performed on ELISA positive samples. A total 675 travel episodes were analyzed. The median age was 25 years and median trip duration was 19 weeks. Half of participants traveled for military exercises, 25% deployed operationally, and 25% traveled for temporary duty assignments. Southeast Asia (25%) and Africa (21%) were the most common destinations. Thirtyeight percent reported using doxycycline for malaria chemoprophylaxis. Posttravel ELISA demonstrated 17% seroprevalence (116/675) and 6.1% seroconversion rate (40/658). Among ELISA seroconverters, 7 (20%) reported subjective fever. No participant met CDC criteria for confirmed or probable leptospirosis. One asymptomatic female SM who traveled to Southeast Asia demonstrated seroconversion with a posttravel titer of 1:200, meeting CDC criteria for presumptive leptospirosis. Additionally, nine participants exhibited lowlevel MAT reactivity (primarily stable 1:100 titers in paired sera), indicating non-specific cross-reactivity or prior exposure. These findings underscore need for sensitive, field-expedient diagnostic tools and enhanced surveillance systems for identifying highrisk subgroups—particularly in heterogeneous, globally mobile military populations where exposures, behaviors, and environmental risks vary widely and are often difficult to characterize.

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LSSSCO-PREP: LEPTOSPIROSIS AND OTHER ZOONOSES, SHIGELLOSIS AND OTHER INFECTIOUS ENTERITIDES, SKIN AND SOFT TISSUE INFECTIONS, INFECTIOUS COUGH, AND OBLIGATE INTRACELLULAR PATHOGEN PRE-EXPOSURE PROPHYLAXIS

Graham Ellis[1], Todd Gleeson[2]

[1]US Navy, Washington, DC, United States, [2]Baltimore Veterans Affairs Medical Center, Baltimore, MD, United States

Doxycycline is a first-line chemoprophylaxis against malaria and commonly prescribed to US military for overseas travel. Its role as post-exposure prophylaxis against anthrax, tularemia, and Lyme disease is established and it is increasingly used as prophylaxis against respiratory and sexually-transmitted infections (STI) in specific populations. For its broad spectrum antimicrobial activity, anti-inflammatory effects, and tolerability, doxycycline is the treatment of choice for multiple respiratory and tropical infections, and its potential as pre-exposure prophylaxis against gastrointestinal (GI), skin and soft tissue (SSTI), and tropical infections has been explored. Optimal chemoprophylaxis against travel-related and tropical infections in southeast Asia is a priority for US military planners and for travel health provision generally. We conducted a retrospective cohort study of 5,328 US servicemembers who were prescribed doxycycline or atovaquone/proguanil as malaria prophylaxis for travel to Thailand of mean duration 45 days. Using ICD-10 codes entered in the one year following return from travel, we describe the incidence of various GI, respiratory, SSTI, STI, and tropical diagnoses. Respiratory diagnoses predominated (918), followed by SSTI (194), and GI (174). The most prevalent diagnosis was "J06.9 Acute URI, unspecified" (345) followed by "R19.7 Diarrhea, unspecified" (222). Few tropical diagnoses were identified, specifically no shigella, leptospirosis, syphilis, Lyme, rickettsia, or malaria; however over 100 STI diagnoses were identified, largely chlamydial. After stratification by type of malaria prophylaxis prescribed (93% having received doxycycline), prescription of doxycycline was associated with a statistically-significant reduced risk of respiratory diagnoses (RR 0.63, 95% CI 0.52-0.75), in both unadjusted and adjusted models. This risk reduction was similar to that observed in prospective observational studies of travelers and adds evidence in support of the prescription of doxycycline for prophylaxis against pathogens other than malaria during international travel.

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LACK OF CONSENSUS ON 'NORMAL BACKGROUND' IN MULTIPLEX SEROLOGY FOR SEROSURVEILLANCE: A SYSTEMATIC METHODOLOGICAL REVIEW

Margaret Dickson[1], May C. Chu[2], Rosemary Rochford[3], Judith Giri[2]

[1]George Washington University - Milken Institute School of Public Health, Washington, DC, United States, [2]Colorado School of Public Health - CU Anschutz, Aurora, CO, United States, [3]University of Colorado Anschutz School of Medicine, Aurora, CO, United States

Accurate serosurveillance requires distinguishing true immunologic signal from baseline healthycontrol reactivity, yet no standard exists for this in serological assays. This heterogeneity complicates cross-study comparisons, increases misclassification risk, and challenges low- and middle-income countries (LMIC), where high background reactivity and limited validated controls hinder interpretation. We conducted a PROSPERO systematic review (CRD420251178089) of serological studies using multiplex magnetic immunoassays (MMIA) to test our hypothesis. Two PubMed searches of human seroepidemiologic studies using multiplex immunoassays were conducted. A PubMed search of human seroepidemiologic studies using multiplex immunoassays identified 29,770 records. After applying restrictions (2010 onward, human studies, multiplex platforms) and excluding non-English publications, reviews, and assay-only studies, 200 studies were retained for full review. 200 studies underwent full screening, 134 were assessed for eligibility, and data were extracted on baseline type, seronegativity definitions, and cutoff methods. Included studies, by baseline type, were categorized as: both internal & external (n=24); internal only (n-80), external only (n=8), and neither (n=22). Results revealed substantial variability: most studies relied solely on internal controls, and among those using external baselines; categorized methods varied with no standard applied. Cutoff determination methods varied, and justification for baseline selection was often limited. The absence of standardized "normal background" definitions impedes comparability and interpretability of serosurveillance data. Developing consensus guidelines and reference materials are needed to improve assay calibration, strengthen reliability of global serosurveillance, and support evidence-based public health decisions, particularly in LMICs.Standardizing baselines will create accurate cross-study comparability and optimize MMIA platforms for near real-time surveillance.

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ANTIMICROBIAL RESISTANCE STEWARDSHIP INTEGRATION WITHIN INFECTIOUS DISEASE PROGRAMS THROUGH AN INTEGRATED QUALITY IMPROVEMENT PLATFORM IN THREE NIGERIAN STATES

Yemisi Bolanle Ogundare[1], Perekebina David Gbabo[1], Samiyah Jumah[1], Ibrahim Nenge[1], Olotu Muriano[1], Adetiloye Oniyire[1], Silvia Kelbert[2], Tigistu Adamu[2], Nochiketa Mohanty[3]

[1]Jhpiego, Abuja, Nigeria, [2]Jhpiego, Baltimore, MD, United States, [3]Jhpiego, New Delhi, India

Fragmented programming across malaria, TB, HIV, and MNCH limits opportunities to strengthen antimicrobial stewardship in routine health systems. In malaria-endemic settings, inconsistent parasitological diagnosis, weak fever classification, and poor pharmacy verification drive inappropriate artemisinin-based combination therapy (ACT) use and unnecessary antibiotic exposure. This study evaluated the integrated quality improvement (QI) platform on diagnostic-led malaria treatment and antimicrobial stewardship in Nigeria.From 2024-2025, multidisciplinary QI teams were supported across 44 LGAs using the WHO Point-of-Care QI methodology in a pre-post analysis. Teams mapped malaria case management processes and identified gaps including treatment before test confirmation, weak documentation, and antibiotic dispensing without positive test results. Through Plan-Do-Study-Act cycles, interventions included test-first workflows, pharmacy verification before dispensing, standardized triage, provider mentorship, and improved documentation. Performance was tracked monthly using DHIS2 indicators and run charts. Rapid diagnostic test use among febrile patients increased from 96.9% (2.30M/2.37M) in 2024 to 99.7% (2.91M/2.92M) in 2025. Inappropriate ACT use in test-negative patients declined from 1.2% (8,153/678,540) to 0.04% (370/983,823). Antibiotic use among confirmed malaria patients without documented co-infection decreased from 14% (10,770/76,938) to 9% (4,993/55,756). Additional gains included improved adherence to treatment guidelines, better documentation, and stronger triage. Integrated QI platform can reduce inappropriate antimicrobial use while strengthening malaria case management and routine health system performance, providing a scalable pathway to advance antimicrobial stewardship and global health security.

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FROM COLORADO TO GUATEMALA: HIGH ANTIMICROBIAL RESISTANCE CHALLENGES EMPIRIC URINARY TRACT INFECTION (UTI) THERAPY AT A DISTRICT HOSPITAL

Philip K. Angelides[1], Roger León[2]

[1]University of Colorado School of Medicine, Aurora, CO, United States, [2]Department of Medicine, Hospital Nacional "Juan José Ortega" de Coatepeque, Coatepeque, Guatemala

Antimicrobial resistance threatens empiric treatment of urinary tract infections (UTIs) in Guatemala. Regional data from a higher-level referral hospital in western Guatemala show 67% ESBL-producing Enterobacterales colonization among hospitalized patients, highlighting the severity of antimicrobial resistance in the region. We analyzed six months of urine culture and susceptibility data (August–December 2025) from inpatient services at Hospital Nacional de Coatepeque, a district-level hospital in western Guatemala. Of 314 cultures, *Escherichia coli* (n=116) and *Klebsiella pneumoniae* (n=42) were the predominant organisms. *E. coli* demonstrated high resistance rates, including 72% to trimethoprim-sulfamethoxazole, 57% to ciprofloxacin, and 48% to ceftriaxone, excluding these agents from reliable empiric use. *K. pneumoniae* showed 52% ceftriaxone resistance and 12% carbapenem resistance. These rates exceed accepted stewardship thresholds for empiric therapy, leaving few reliable oral options. We additionally assessed antibiotic formulary availability, stock variability, and cost to inform real-world prescribing constraints. Using a U.S. hospital UTI protocol as a framework, we developed a context-adapted empiric algorithm integrating resistance patterns with formulary constraints. Preliminary recommendations include nitrofurantoin for uncomplicated UTI, with limited reliability of ceftriaxone in complicated infections and early escalation strategies for suspected ESBL-producing Enterobacterales. The algorithm was designed for low-resource clinical workflows. This first district-level antibiogram highlights the urgent need for facility-specific empiric protocols in resource-constrained settings where standard guidelines may not apply and resistance patterns are rapidly evolving.

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EXPANDING THE GLOBAL HEALTH PIPELINE: A COLLABORATIVE, CROSS-LEVEL APPROACH TO MEDICAL EDUCATION.

Kristina Krohn[1], Adriana Dhawan[2], Megan K. Shaughnessy[3], Patricia F. Walker[4], Bobbi Sue Pritt[5], Brett R. Hendel-Paterson[6], Stephen Dunlop[7], Wasin Matsee[8], Paul Buyego[9], Nathan Bertelson[2], Alberto Matteelli[10], William Stauffer[11]

[1]Univ of Minnesota, Roseville, MN, United States, [2]Univ of Minnesota, Minneapolis, MN, United States, [3]University of Minnesota, Medical School, Minneapolis, MN, United States, [4]Global Medicine, Dept of Medicine, Saint Paul, MN, United States, [5]Mayo Clinic, Rochester, MN, United States, [6]University of Minnesota, Mendota Heights, MN, United States, [7]Hennepin County Medical Ctr, Columbia Heights, MN, United States, [8]Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand, [9]Infectious Disease Institute, Kampala, Uganda, [10]University of Brescia, Brescia, Italy, [11]Univ of Minnesota Dept of Medicine, Minneapolis, MN, United States

Since 2005, the University of Minnesota's Clinical Global Health and Tropical Medicine Curriculum (CGHTM) has transformed into a highly interactive,

internationally networked, global learning platform. Founded in 2005, it has expanded through global partnerships with the Centers for Disease Control and Prevention, Mayo Clinic, Mahidol University in Bangkok, University of Ottawa, Makerere University in Uganda, and University of Brescia in Italy. Together we created a worldwide accessible curriculum through 7 asynchronous online courses, and attendance at one of four in-person 2-week courses. Recent work has focused on developing curated and interactive digital lessons, many co-created by students working directly with subject matter experts - an intentional model that simultaneously builds workforce capacity in global health and in educational design. Between 2015 and 2025, 694 participants took an in-person course, 481 in Minnesota, 137 in Thailand, 61 in Uganda, and 19 in Italy. Participants from all 50 states, the District of Columbia, Puerto Rico and 55 countries have taken an online and/or in-person course. During 2020-2022, medical students took 528 of the online one-week courses. Since 2016 more than 1400 people enrolled in any of the courses. Approximately 1/3 (233/639) of those who received a CTropMed® from 2010 through 2023 qualified through our curriculum. Over twenty years, the curriculum has moved from a focus on tropical diseases to a broad, equity-driven approach addressing power structures, forced migration, trauma, decolonization, diseases of disparities, and migrated to interactive, flexible, learner-centered online lessons. Adding to our accreditation from the American Society of Tropical Medicine in the United States, Minnesota's CGHTM is also now recognized in the United Kingdom (DTM&H) and in Europe (TropEd Network). Next steps include deepening adult-learning strategies, elevating partner faculty voices, and delivering this essential knowledge to more institutions.

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EARLY DYNAMICS OF POWASSAN VIRUS INFECTION IN HUMAN SKIN

Alex Segura[1], Aleena Alex[1], Jacob Blackledge[2], Isabelle Kahng[1], Simon Barratt-Boyes[1], Danielle M. Tufts[1], **Priscila MS Castanha**[1]

[1]University of Pittsburgh, Pittsburgh, PA, United States, [2]Pittsburgh Science and Technology Academy, Pittsburgh, PA, United States

Powassan virus (POWV) is an emerging tick-borne *Orthoflavivirus* transmitted by *Ixodes scapularis* that can cause severe neuroinvasive disease, with nearly half of survivors experiencing long-term neurological sequelae. Despite its public health importance, the early stages of POWV infection in human skin and the modulatory role of tick saliva remain poorly understood. To investigate these early dynamics, we inoculated human skin explants subepidermally with 6 logs of POWV (DTV Spooner) in the presence or absence of *I. scapularis* salivary gland extract (SGE). Tissue and emigrated cells were collected at multiple time points. Viral replication was assessed via confocal microscopy for the NS3 protein and cell-specific markers, while viral load was quantified using RT-qPCR. We found that POWV efficiently infects and replicates within both the epidermal and dermal layers, but with distinct kinetics. In the absence of SGE, epidermal infection primarily targeted the basal keratinocyte layer, with peak replication occurring at 120 hours post-inoculation (hpi), while dermal infection peaked earlier at 72 hpi. Notably, while POWV infection triggered a robust recruitment of dermal macrophages to the inoculation site, these cells showed negligible infection. This suggests that macrophages are not primary targets for POWV in human skin, a feature that distinguishes it from other *Orthoflaviviruses* such as dengue and Zika. Cellular migration out of the skin was significantly higher in POWV-infected tissue compared to mock-infected controls at all time points. Notably, the addition of tick SGE significantly enhanced both viral replication (1-log increase) and cellular emigration from the skin (3-fold increase). The detection of viral RNA in the migrated cells suggests these populations may facilitate systemic dissemination to lymph nodes. This foundational model provides proof-of-concept that human skin is highly susceptible to POWV and offers a robust platform to study the early microenvironment of POWV infection and tick-host-pathogen interactions.

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DEVELOPMENT AND CHARACTERIZATION OF A SUBLETHAL-SEQUELAE MOUSE MODEL OF EASTERN EQUINE ENCEPHALITIS VIRUS INFECTION

Shannon K. Carney[1], Caitlin Woodson[1], Elizabeth Harris[1], Morgen VanderGiessen[1], Caroline de Jager[1], Tanya LeRoith[1], Barney Bishop[2], Michelle Theus[1], Kylene Kehn-Hall[1]

[1]Virginia Tech, Blacksburg, VA, United States, [2]George Mason University, Manassas, VA, United States

Eastern equine encephalitis virus (EEEV) is a mosquito-borne encephalitic alphavirus that causes disease in equines and humans with a 50-75% mortality rate and, among the survivors, 50-90% experience debilitating neurological sequelae. Despite this burden, no therapeutics exist, few studies have described EEEV pathogenesis in lethal mouse models, and no model currently exists to study long-term neurological sequelae. To address this gap, we aim to characterize neuroinflammatory and cognitive outcomes following infection and *hypothesize* that defining transcriptomic, neuropathologic, and cognitive changes in mice following EEEV infection will establish a sublethal-sequelae mouse model of EEEV infection that can be used for therapeutic approval. To enable survival, EEEV FL939-39 (EEEV WT) was mutated in three virulence-associated regions, and a nano luciferase reporter was inserted to allow longitudinal tracking of viral replication (EEEV TM-nLuc). C57BL/6 mice were intranasally infected with 10^3 plaque-forming units (PFU) of WT-nLuc or 10^5 PFU of TM-nLuc, in vivo imaged up to day 28 post-infection (pi), and brains collected at days 4, 8, or 90pi. Imaging revealed that EEEV TM-nLuc replication peaked at d8pi and persisted until d21pi. By d8pi, neuronal necrosis, meningitis, perivascular cuffing, and gliosis were observed in the brains of EEEV TM-nLuc-infected mice, with meningitis, perivascular cuffing, and gliosis persisting to d28pi. Cognitive and behavioral testing (Elevated Plus Maze, modified SHIRPA test, Novel Object Recognition) at days 30, 60, and 90pi showed persistent neuromuscular deficits; cognitive analyses are ongoing. These data indicate TM-nLuc establishes a persistent, neuroinflammatory infection enabling study of long-term neuropathology and functional sequelae. Ongoing studies include assessment of hippocampal immunohistochemistry and single-cell RNA sequencing to define transcriptomic alterations. This model will characterize the host response to EEEV infection and understand the associated neuropathology and potential therapeutic targets to prevent neurological damage and disease.

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SUBTLE NEUROBEHAVIORAL IMPAIRMENTS AND SYNAPTIC VESICLE PATHWAY DISRUPTION FOLLOWING MILD NEONATAL ZIKA VIRUS INFECTION IN MICE

Jaime Alexander Chivatá-Avila[1], Paola Rojas-Estevez[2], Aura Caterine Rengifo[2], Jose Manuel Lozano[1], Diego Alejandro Alvarez Diaz[2]

[1]National University of Colombia, Bogotá, Colombia, [2]National Institute of Health Colombia, Bogotá, Colombia

Zika virus (ZIKV) has been associated with a broad spectrum of neurodevelopmental outcomes, ranging from congenital malformations to milder cognitive and behavioral disturbances that may remain undetected during early life. These subtle manifestations have become increasingly relevant in public health settings, particularly in infants exposed to ZIKV without overt microcephaly. To investigate the mechanisms underlying these milder phenotypes, we established a neonatal BALB/c mouse model of low-dose ZIKV infection using a 2016 epidemic strain. Newborn mice were inoculated subcutaneously on postnatal day one and monitored for viral persistence in the cortex and cerebellum at 10 and 30 days post-infection using RT-qPCR. At 30 days post-infection, neurobehavioral performance was evaluated using the T-maze, rotarod, and open-field paradigms. RNA-sequencing was subsequently performed to characterize transcriptional changes associated with behavioral outcomes. Differentially expressed genes underwent Gene Ontology, KEGG pathway enrichment, and network analysis to identify affected biological processes. Mice survived infection

and exhibited detectable viral RNA in brain tissue. Although locomotor and motivational performance remained intact, infected animals showed significant working memory impairment and increased anxiety-like behavior. Transcriptomic analysis revealed altered expression of genes involved in immune signaling, cytoskeletal regulation, and synaptic vesicle cycling, suggesting long lasting molecular effects despite apparent recovery from infection. These findings indicate that even mild neonatal ZIKV exposure can lead to persistent cognitive alterations accompanied by disruption of pathways critical for neuronal communication and plasticity. This work provides mechanistic insight into the subtle neurological consequences observed in children exposed to ZIKV in utero and underscores the importance of investigating non-severe infection outcomes in emerging neurotropic viruses.

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PERIPHERAL CHIKUNGUNYA VIRUS INFECTION INDUCES PERSISTENT NEUROLOGICAL DISEASE IN AN IMMUNOCOMPETENT, DEVELOPMENTALLY RELEVANT MOUSE MODEL

Freedom Marie Green, Alyssa Lantz, Amanda Cowan, Reina Saldivar, Tori Baxter

Texas Biomedical Research Institute, San Antonio, TX, United States

Chikungunya virus (CHIKV) infection is an emerging global public health concern increasingly associated with neurological disease, yet the mechanisms driving persistent neurological dysfunction remain poorly understood. Progress has been limited by the lack of immunocompetent small-animal models that develop neurological disease following peripheral infection. To address this gap, we investigated clinical disease outcomes in 4- to 6-week-old CC041 mice, a genetically diverse Collaborative Cross strain that supports CHIKV neuroinvasion after peripheral inoculation. CC041 mice were infected via subcutaneous footpad inoculation and longitudinally evaluated for weight change and neurological disease through 28 days post-infection using an adapted, blinded neurological scoring system. Despite the absence of weight loss or mortality, CHIKV-infected CC041 mice developed progressive clinical signs consistent with neurological dysfunction, whereas mock-infected controls remained clinically normal. Approximately half of infected mice reached moderate disease by 10 days post-infection, and all infected mice exhibited sustained neurological signs at later time points. Observed phenotypes included abnormal tail posture, hunching, ataxia, gait abnormalities, and pronounced hyperactivity. Neurological signs were consistent with functional impairment of brain regions in which viral antigen was detected by immunohistochemistry at early time points (~5 days post-infection), including the cerebellar nuclei. Notably, neurological abnormalities persisted well beyond clearance of infectious virus from the brain, suggesting that post-acute mechanisms contribute to ongoing disease. Together, these findings demonstrate that peripheral CHIKV infection can drive prolonged neurological disease in an immunocompetent, developmentally relevant mouse model, providing a valuable platform for studying post-viral neurological sequelae and evaluating therapeutic interventions.

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EMERGING STRAINS OF CACHE VALLEY VIRUS DEMONSTRATE INCREASED NEUROPATHOGENESIS IN MURINE MODELS

Albert Jonathan Auguste[1], John A. Muller[1], Manette Tanelus[1], Dawn I. Auguste[1], Avery Anna Kaye[2], Elise Hennessy[2], Maranda Stricklin[1], Campbell D. Winn[1], Krisangel Lopez[1], Chad Mire[3], Alexander T. Ciota[4]

[1]Virginia Tech, Blacksburg, VA, United States, [2]Blacksburg, VA, United States, [3]USDA, ARS, NBAF, Manhattan, KS, United States, [4]Wadsworth Ctr, NY State Dept of Hlth, Slingerlands, NY, United States

Cache Valley Virus (CVV) is an emerging mosquito-borne orthobunyavirus of significant concern to livestock and human health. CVV strains are phylogenetically classified into two genetic lineages. Recent surveillance studies indicated that lineage II strains originating from Mexico have displaced lineage I isolates in the Northeast USA, since 2010. Furthermore, reassortant isolates have emerged and are associated with several documented human clinical cases. To gain a better understanding of lineage-specific differences in pathogenesis, we explored the pathogenesis among three CVV strains from lineage I, lineage II, and reassortant viruses in immune-competent and immune-compromised murine models, with an emphasis on neuropathogenesis. Our results show significant strain-specific differences in pathogenesis with clear differences in weight loss, viremia, organ loads, and mortality. Considering our comparative pathogenesis data as a surrogate for understanding differences in human pathogenesis, our data suggests increased neuropathogenesis among the lineage II strains and one of the reassortant viruses studied. Our studies also identify a unique, highly neurovirulent reassortant CVV strain that presents significantly more disease than lineage I and II strains in several neurovirulence models. Interestingly, no differences in *in vitro* replication dynamics were observed among lineages, except for the highly neuropathogenic reassortant strain. Altogether, our results suggest significant strain- and lineage-specific differences in CVV neuropathogenesis and suggests a plausible increased risk of neurological disease may be associated with currently circulating lineage II strains. CVV remains an important agricultural and human pathogen and further research is needed to understand the factors that contribute to its risk of emergence and disease burden.

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ASSESSING NEUROVIRULENCE OF CONTEMPORARY POWASSAN VIRUS STRAINS IN A MOUSE MODEL

Julia C. Pritchard[1], Grace J. Vaziri[1], Jordan Mandli[2], Hannah Theriault[3], Alan Philip Dupuis[4], Alexander T. Ciota[4], Matthew Aliota[1]

[1]University of Minnesota, Saint Paul, MN, United States, [2]Minnesota Department of Health, Saint Paul, MN, United States, [3]State University of New York - Albany, Albany, NY, United States, [4]Wadsworth Center-New York State Department of Health, Slingerlands, NY, United States

Human Powassan virus (POWV) infections are increasing each year in the Northeast and Upper Midwest. Most reported cases are severe, with CNS involvement, approximately 10% fatality, and long-term neurological impact in 50% of survivors. Many POWV studies use historic, highly passaged strains. Therefore, we sought to assess the capacity of contemporary virus strains to cause clinical disease. We assessed virulence of genetically and geographically distinct POWV strains isolated from ticks in MN and NY. Six-week-old C57BL/6 mice (n=8 per group) were subcutaneously inoculated with 1000 PFU of tick-derived lineage I (n=2, NY) and lineage II (n=2, NY; n=2, MN) viruses. This dose approximates the amount of virus delivered by a feeding tick. All mice were productively infected, with virus replication confirmed in the blood or brain. Viremia peaked early and resolved by 6 days post infection (DPI) in all groups. Early viremia differed between virus groups at 1 and 2 DPI but was comparable by 3 DPI. Mice that succumbed to infection met euthanasia criteria between 7-11 DPI. Survival differed between virus groups (Mantel-Cox test, $p < 0.0001$; 0-75% survival) and was not associated with lineage. All mice that succumbed to infection had infectious virus present in the brain at time of euthanasia (5.78-9.68 log10 PFU/g). Infectious virus was also detected in most surviving mouse brains at the experimental endpoint (21 DPI, 0-3.55 log10 PFU/g). Brain viral titers differed significantly by survivor status for each group (Welch's t-test, $p < 0.01$) . We are additionally characterizing neutralizing antibody responses in survivors, virus diversity in the brain, and systemic infection through visceral organs. These findings emphasize the role of POWV genetic variability on disease heterogeneity and are important for understanding the future trajectory and public health impact of POWV.

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INFECTION-MEDIATED TRAIT VARIATION ALTERS TEMPERATURE-DEPENDENT TRANSMISSION OF WEST NILE VIRUS

Rachel Lucy Fay[1], Mauricio Cruz-Loya[1], Elyse Mary Banker[2], Erin Mordecai[3], Alexander T. Ciota[4]

[1]Stanford University, Stanford, CA, United States, [2]NYSDOH, Slingerlands, NY, United States, [3]Stanford, CA, United States, [4]Wadsworth Ctr, NY State Dept of Hlth, Slingerlands, NY, United States

Arthropod-borne viruses are responsible for more than 140 human diseases worldwide, with the most widespread belonging to the flavivirus family, including West Nile virus (WNV). Rising global temperatures are reshaping species interactions and altering the ecological conditions that govern vector-borne disease transmission. While previous studies have shown that WNV infection can modify mosquito longevity, fecundity, and blood-feeding behavior, trait-based R_0 models have largely relied on data from unexposed mosquitoes, implicitly assuming homogeneous vector populations. This assumption overlooks infection-induced heterogeneity that may scale up to influence transmission dynamics. Here, we examine how temperature, infection status, and viral strain interact to shape transmission potential in the mosquito vector *Culex pipiens*. Life-history traits of WNV-exposed and unexposed mosquitoes were quantified across constant temperatures from 10°C to 33°C, as well as under a diurnal cycling regime centered around 25°C. These empirical data were used to generate thermal performance curves and estimate relative R_0 across treatments. We demonstrate that infection status interacts with temperature to alter mosquito life-history traits, vector competence, and overall transmission potential. Including infection-sensitive traits into relative R_0 calculations substantially reduced estimated transmission intensity across much of the thermal range. Our findings highlight the ecological importance of within-vector heterogeneity and suggest that models excluding infection-mediated trait variation may overestimate transmission under warming scenarios. By integrating infection-sensitive traits into temperature-dependent frameworks, this study advances ecologically grounded predictions of WNV dynamics and underscores the need to incorporate infection-driven heterogeneity into climate-driven disease models.

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DECIPHERING MOSQUITO DETERMINANTS OF ARBOVIRUS VERTICAL TRANSMISSION IN *AEDES AEGYPTI*

Gladys Gutierrez-Bugallo, Cindy Megumi Kimura, Shengzhang Dong, George Dimopoulos

Johns Hopkins University Bloomberg School, Baltimore, MD, United States

Arthropod-borne viruses have affected humans for millennia and continue to cause epidemics through emergence and re-emergence events. *Aedes aegypti* is the primary vector responsible for major outbreaks. While horizontal transmission (HT) between mosquitoes and humans drives most infections, vertical transmission (VT) from mother to offspring is considered an evolutionary mechanism that enables viral persistence during periods unfavorable for HT. Vertically infected females can also excrete infectious virus in saliva without biting an infected host, potentially contributing to virus re-emergence. To investigate mosquito determinants of VT, we performed assays infecting a laboratory *Ae. aegypti* colony with dengue virus type 2 (DENV-2) and Mayaro virus (MAYV). Viral dissemination to the head and ovaries of exposed females was assessed by plaque assay, and infection in progeny was measured by RT-qPCR. VT rates were estimated for each virus/mosquito combination. We found no statistically significant differences in infection rates between heads and ovaries for either virus. However, VT rates were significantly lower than ovary infection rates, suggesting intrinsic factors in the ovary may limit virus inheritance to offspring. A subset of the progeny was positive for each virus, raising questions about what determines susceptibility or refractoriness to infection at this stage. Differential gene expression analyses identified potential candidate genes associated with susceptibility to vertical transmission. These findings provide insights into the mosquito factors influencing VT, contributing to our understanding of arbovirus persistence in nature and potential mechanisms underlying virus re-emergence.

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TEMPERATURE EFFECTS ON VECTOR COMPETENCE OF JAPANESE ENCEPHALITIS VIRUS IN TWO MOSQUITO SPECIES

Faustus Akankperiwen Azerigyik[1], Kaleb Davis[2], William C. Wilson[3], Dana Mitzel[4], Rebekah Kading[1]

[1]Colorado State University, Fort Collins, CO, United States, [2]Fort Collins, CO, United States, [3]USDA, ARS, Manhattan, KS, United States, [4]US Department of Agriculture (USDA) Agricultural Research Service (ARS), Manhattan, KS, United States

Temperature is an important driver of vector competence and transmission dynamics for Japanese encephalitis virus (JEV). Since JEV is a virus at risk of introduction to the United States, we sought to compare the response to temperature in two important domestic mosquito vector species: *Culex tarsalis* and *Cx. quinquefasciatus*. Experimental infections were performed at 18°C, 28°C and 32°C constant temperatures, in which both species were fed a virus-spiked blood meal ($1.5x10^6$ pfu/mL, JEV genotype 1). Vector competence parameters including infection (IR), dissemination (DR), and potential transmission (TR) rate were assessed at 7 and 14 days post infection (dpi) using RT-qPCR and plaque assays to capture species-specific differences.Our preliminary data showed distinct competence profiles at 28ºC and 70% humidity, with significantly high IR, 100%, and DR of >80%, consistent with detectable viral loads after 7dpi in both *Cx. tarsalis* and *Cx. quinquefasciatus*. Interestingly at 14dpi, *Cx. tarsalis* showed higher IR, 100% and DR, 80%, yet demonstrated a lower TR of 40%. In contrast, *Cx. quinquefasciatus* showed an IR of 95%, and DR of 65%, with a higher TR of 65%, suggesting a salivary gland barrier to transmission in *Cx. tarsalis*. Our ongoing studies continue to evaluate temperature variability by comparing exposure to thermal extremes (~18 and 32 ºC) on vector competence, viral diversity and mosquito immune responses, with the expectation that incorporating realistic temperature variations will reveal potential shifts in vector competence not captured under permissive conditions.Importantly, this study demonstrates that vector competence for JEV is strongly influenced by the mosquito species under equivalent temperature conditions, and that *Cx. tarsalis* has a moderate salivary gland barrier compared to *Cx. quinquefasciatus* for JEV genotype 1. The differential responses observed between *Cx. tarsalis* and *Cx. quinquefasciatus*, even under permissive thermal conditions shows how temperature can reshape the transmission risk and epidemiology of Japanese encephalitis virus.

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WHY HAS IT BEEN SO DIFFICULT? SUCCESSES AND LIMITATIONS OF FIFTY YEARS OF IMMUNOLOGICAL STUDIES TO IDENTIFY ANTIGENIC TARGETS, BIOMARKERS, AND MECHANISTIC CORRELATES OF PROTECTION INDUCED BY WHOLE *PLASMODIUM FALCIPARUM* SPOROZOITE VACCINES

Stephen L. Hoffman

Sanaria Inc, Rockville, MD, United States

Immunization with attenuated *Plasmodium falciparum* (Pf) sporozoites (SPZ) by mosquito bite or injection provides the most effective protection of any immunogen against controlled human malaria infection or field transmission in adults, including pregnant women. Recently, high level protection has been achieved in infants born to mothers immunized pre-conception. Since the 1st demonstration in the early 1970s of protection of humans by PfSPZ, there have been intense efforts to determine the antigenic targets and immunological mechanisms underpinning this protection to inform subunit vaccine and improved vaccine delivery systems development and predict who will be protected. Studies in the 1970s/1980s implicated IgG antibodies to the PfCSP repeat region, providing the rationale for

development of RTS,S, R21, and anti-PfCSP monoclonal antibodies. By the late 1980s, it was clear that $CD8^+$ T cells and IFN-γ targeting unknown antigens in *Plasmodium*-infected livers were required for SPZ-induced protection. In the 1990s, TRAP/SSP2, HEP17/EXP1, PfLSA1, PfLSA3 and other antigens were implicated as targets of protective immunity, and nitric oxide, IL-12 and NK cells as mediators in the pathway of protection. Subsequently, genomics, transcriptomics, proteomics, refined techniques for assessing cellular immunology including TCR sequencing, and systems immunology have provided a plethora of data implicating IgM and IgA antibodies, antibody-Fc receptor binding dynamics, NK T cells, γδ T cells, and other antigens including PfCelTOS and RPL6 in pre-erythrocytic stage protection. However, few antigens have made it to clinical trials and none have been shown to confer significant protection in humans, and none of the assays have identified a biomarker or immunological correlate of protection at the individual level. The existing literature, unpublished data, potential reasons for lack of success to date, and initial results of an approach using machine learning and AI to integrate all efficacy and immunology findings taking into account genetic background, age, previous malaria parasite exposure, and parasite origin will be presented.

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BEYOND THE MOSQUITO: ESCAPING THE MIDGUT BOTTLENECK FOR IPFSPZ VACCINE PRODUCTION

Tales Pascini[1], Ehud Inbar[1], Franck Dumetz[2], Minglin Li[1], Tint Tint Wai[1], Asha Patil[1], Yonas Abebe[1], David Serre[2], Sumana Chakravarty[1], B. Kim Lee Sim[1], Stephen L. Hoffman[1]

[1]Sanaria Inc., Rockville, MD, United States, [2]University of Maryland School of Medicine, Baltimore, MD, United States

Sanaria® PfSPZ vaccines are composed of *Plasmodium falciparum* (Pf) sporozoites (SPZ) produced in aseptic *Anopheles stephensi* mosquitoes (mPfSPZ). Transitioning to *in vitro* production (iPfSPZ) is critical for a low-cost vaccine for malaria elimination. However, current iPfSPZ are qualitatively distinct from mPfSPZ, having smaller oocysts with different gene and protein expression, suggesting a lack of vector signals. We performed comparative transcriptomic profiling (RNAseq) of Pf at 2h, 24h, and 3 days post-infection (dpi) *in vivo* and *in vitro*. Development was assessed using Pearson correlation (r) comparing samples with ookinete profile (Malaria Cell Atlas), and validated using stage-specific markers (Pfs25, PfCAP380, Pfs48/45, PfCSP, and PfChitinase) by qRT-PCR and confocal microscopy. At 2h, *in vivo* and *in vitro* samples showed similar profiles (r=0.21-0.24, P<0.001). At 24h, expression also correlated (r=0.40-0.49, P<0.001). Significant differences emerged by 3 dpi: *in vivo* parasites successfully transitioned to oocysts, showing a loss of ookinete identity (r=0.11, P<0.001), while *in vitro* parasites remained transcriptionally locked in an ookinete-like state (r=0.38, P<0.001). At 8 dpi, parasites that further develop *in vitro* formed smaller oocysts (~30µm) *vs in vivo* (~60µm) with distinct PfCSP expression patterns. Our data suggest *in vitro* cultures lack vector signals, resulting in "homeostatic arrest"; many parasites stall as ookinetes. RNAseq also revealed a significant upregulation of the *IK2* translational brake (log_2FC>3.5) and metabolic stress pumps (ABCG2) initiated at the 24h-48h window, with failure to activate *AQP* and *PIMMS2*. At 3 dpi, a >10-fold deficit in *AQP* transcripts was observed *in vitro* compared to *vivo* controls. The *in vitro* stagnation observed is a "transcriptomic trap" triggered after the 24h ookinete peak—a stage normally eliminated by the midgut bottleneck *in vivo*. We propose that by overriding the *IK2* brake and activating AQP through synchronized environmental and hormonal cues, we may successfully drive oocyst maturation and syncytium formation for iPfSPZ production.

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IDENTIFICATION OF POTENT NEUTRALIZING ANTIBODIES AGAINST *PLASMODIUM FALCIPARUM* CIRCUMSPOROZOITE PROTEIN (CSP) FROM NATURALLY INFECTED DONORS

Adriano Franco[1], Cherrelle Dacon[1], Andrew J R Cooper[1], Jake Kritzberg[1], Courtney Tucker[1], Samantha Petros[1], Shanping Li[2], Safiatou Niare Doumbo[3], Didier Doumtabe[3], McAleese Holly[4], Pena Mirna[5], Alvaro Molina-Cruz[6], Kassoum Kayentao[3], Aissata Ongoiba[3], Boubacar Traore[3], Barillas-Mury Carolina[6], Peter D. Crompton[2], Joshua Tan[1]

[1]Antibody Biology Unit, Laboratory of Immunogenetics, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States, [2]Malaria Infection Biology and Immunity Section, Laboratory of Immunogenetics, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States, [3]Mali International Center of Excellence in Research, University of Sciences, Technique and Technology of Bamako, Bamako, Mali, [4]Comparative Medicine Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States, [5]Laboratory of Immunogenetics, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States, [6]Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States

Monoclonal antibodies (mAbs) are one of the most promising strategies for the prevention of infectious diseases. The three most clinically advanced mAbs against *Plasmodium falciparum* sporozoites (CIS43, L9, and MAM01) were all isolated from malaria-naïve individuals enrolled in malaria vaccine clinical trials and target the circumsporozoite protein (CSP) of the parasite. The development of sporozoite-specific mAbs during natural infection and the naturally acquired antibody response against *Pf*CSP are understudied and could prove pivotal in the identification of a potent candidate that complements WHO-recommended vaccines. Here, we screened plasma samples from 751 Malian donors with evidence of previous malaria exposure to identify antibody reactivity to CSP. We individually isolated memory B cells (MBCs) from 58 highly reactive donors and used a high-throughput flow-cytometry approach to assess their reactivity to recombinant *Pf*CSP and *Pf*CSP-derived peptides. We further downselected the 27 most reactive donors for a monoclonal B cell screen using an optofluidics system. Individual MBCs that were reactive to *Pf*CSP were selected for heavy and light chain PCR amplification, sequencing, and antibody production. The first 41 mAbs were validated in binding assays to *Pf*CSP and *Pf*CSP-derived peptides and tested in mice to assess their neutralizing capacity against a sporozoite challenge. Our current most potent neutralizing mAbs, ABU4-004 and ABU4-007, decrease parasite liver burden at similar levels to current best-in-class mAbs. Future work will focus on the comparison of antibody responses to *Pf*CSP after vaccination and infection, as well as finding antibody correlates of protection against sporozoites.

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CRYO-EM ANALYSIS OF A LARGE PANEL OF PFCSP-REACTIVE HUMAN ANTIBODIES LEADS TO STRUCTURE-BASED DESIGN OF NEW, HOMOTYPIC ANTIBODIES FROM GERMLINE SEQUENCE WITH IMPROVED PROTECTION IN VIVO

Fabien Cannac[1], Wilma Lee[1], Monika Jain[1], Johannes Loeffler[1], Flores-Garcia Yevel[2], Nathan Beutler[1], Fidel P. Zavala[3], Monica Quintero[1], Gregory J. Martin[4], Ian A. Wilson[5], Andrew B. Ward[1]

[1]Scripps Research, La Jolla, CA, United States, [2]JHU, Baltimore, MD, United States, [3]Johns Hopkins Univ Sch of Pub Hlth, Baltimore, MD, United States, [4]US Department of State, Washington, DC, United States, [5]The Scripps Research Institute, La Jolla, CA, United States

Over the past decade, several antibodies binding to the *Plasmodium* circumsporozoite protein (CSP) have been shown to form homotypic contacts, where multiple copies of the same antibody bind to the

antigen while also forming rich antibody-antibody interactions. We used cryo-electron microscopy (cryo-EM) to look at more than 90 anti-CSP antibodies from more than two dozen lineages and different chain pairings, of which nearly 60% formed multivalent homotypic complexes with the malarial antigen. We were able to sort these into different homotypic superstructures, based on the overall fold adopted by the CSP-antibody complexes, and further show that homotypic interactions observed with Fabs can be conserved as IgG. We used these data to alter the homotypic state of different antibodies and induce for the first time a homotypic conformation from a germline residue, through minimal mutations and leading to increased potency in vivo. We provide in-depth analysis of the relation between the different homotypic phenotypes observed for these antibodies and their published functional and inhibitory data, bringing a new dimension to the understanding of anti-CSP vaccine response and antibody design.

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DURABLE IMMUNITY TO SECONDARY CHALLENGE WITH *ANCYLOSTOMA CEYLANICUM* HOOKWORMS PERSISTS UP TO 1 YEAR FOLLOWING PRIMARY INFECTION

Christina Paraggio[1], Jacquelyn Kauffman[2], Richard Bungiro[2], Grace Singer[2], Martina Wade[2], Michael Cappello[2]

[1]Yale University, New Haven, CT, United States, [2]Yale School of Public Health, New Haven, CT, United States

Acquired resistance to re-infection has been reported in animal models of hookworm infection. We investigated the durability of protection 1-year following primary infection with *Ancylostoma ceylanicum.* Groups of hamsters were uninfected (UN), infected with 50 larvae (INF), or infected and treated with the anthelminthic mebendazole (INF+MEB) at 42 days post infection (DPI). Cure was verified by a cessation in fecal egg excretion. Primary infection (INF and INF+MEB groups) resulted in growth delay and decreased hemoglobin levels, which resolved in the treated group by 56DPI (hemoglobin) and 91DPI (growth). Hemoglobin levels recovered in the INF group by 98DPI, but growth delay was observed through 365DPI. Robust IgG responses to hookworm excretory/secretory proteins (ES) were detected in both infected groups, peaking at 49DPI in the INF+MEB group and at 56DPI in the INF group. Anti-ES IgM peaked at 49DPI (INF+MEB) and 84DPI (INF). Fecal IgA (sIgA) responses to ES were detected by 21DPI, corresponding to the establishment of patent infection in the gut. In the treated group (INF+MEB), sIgA peaked 7 days post treatment, then decreased 43% by 63DPI . Anti-ES sIgA in the INF group peaked 105DPI and remained higher than the INF+MEB group until 273DPI. At 365DPI, all 3 groups were challenged with 200 *A. ceylanicum* larvae to assess susceptibility to re-infection. Compared to the previously uninfected group, serum and mucosal antibody responses to ES were boosted in animals receiving the secondary challenge. At 22 days post challenge, mesenteric lymph nodes in the previously uninfected group (UN) showed a significantly higher average weight (99.8mg +/- 29.6) than the INF (29.7mg +/- 2.1) and INF+MEB (29.8mg +/- 2.6) groups, suggesting an active immune response to adult worms. A total of 121 hookworms were recovered from the previously uninfected group (average per animal 20.2 +/- 9.5), 0 were recovered from the INF group, and 2 were recovered from the INF+MEB group (0.33 +/- 0.2). These data confirm that, in the hamster model, resistance to secondary challenge following primary hookworm infection is highly durable and persists >300 days after deworming.

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ESTIMATED INCIDENCE RATES OF *PLASMODIUM FALCIPARUM* INFECTION ACROSS SUB-SAHARAN AFRICA: THE MAINZ COHORT STUDY

John J. Aponte[1], Ayola Akim Adegnika[2], Pedro C.P. Aide[3], Abel Kakuru[4], Brenda Apio Okech[5], Ally Olotu[6], John P. A. Lusingu[7], Jean Ronald Edoa[2], Armand Martial Keufouet Wamba[2], Wilson Simone[3], Arlindo Chidimatembue[3], Moses R. Kamya[4], Joaniter I. Nankabirwa[4], Mary Muhindo[4], Juliet Mpendo[5], Nasimu Kyakuwa[5], Nicolaus Gutapaka[6], Muanuzi Mutabuzi[6], Sylvester Simon Simando[6], Samwel Gesase[7], Daniel T. R. Minja[7], Joyce R. Mbwana[7], Anangisye Malabeja[7], Inga Tschoepe[8], Joseph Norman[8], Taous Chermak[9], Ramona Mond[10], David M. Schellenberg[11], Pedro L. Alonso[10], Federico J. Mensa[9], Thierry Rolling[10]

[1]BioNTech Switzerland GmbH, Basel, Switzerland, [2]Centre de Recherches Médicales de Lambaréné (CERMEL), Lambaréné, Gabon, [3]Centro de Investigação em Saúde de Manhiça (CISM), Maputo, Mozambique, [4]Infectious Diseases Research Collaboration (IDRC), Kampala, Uganda, [5]Uganda Virus Research Institute-Clinical Research Program (UVRI-CRP), Entebbe, Uganda, [6]Ifakara Health Institute (IHI), Bagamoyo, United Republic of Tanzania, [7]National Institute for Medical Research (NIMR), Tanga, United Republic of Tanzania, [8]Elderbrook solutions, Biberach an der Riß, Germany, [9]BioNTech US Inc, Cambridge, MA, United States, [10]BioNTech SE, Mainz, Germany, [11]BioNTech UK Ltd, London, United Kingdom

Plasmodium falciparum remains a major cause of morbidity and mortality in sub-Saharan Africa, yet age-stratified incidence data for planning next-generation vaccine trials, particularly in non-pediatric age groups, are limited. We conducted a prospective, observational cohort study (MAINZ; BNT000-003) to estimate site- and age-specific incidence rates of *P. falciparum* infection and compare malaria rapid diagnostic tests (mRDT) and expert microscopy of Thick Blood Slides (TBS) against quantitative PCR (qPCR) in six sites representing different malaria transmission intensities in Gabon, Mozambique, Tanzania, and Uganda. We enrolled 2,098 residents aged 5-60 years between April and July 2025 and followed them for 6 months. Active detection of infection consisted of weekly finger-prick mRDTs over 4 months plus a visit at Month 6. TBS and qPCR samples were collected at baseline, Month 2 and Month 6. Incident *P. falciparum* infection was defined as a new mRDT-positive result in participants negative at baseline or ≥4 weeks after a prior positive test. Participants with positive mRDT and/or TBS were treated using in-country treatment guidelines. Passive surveillance at local facilities captured additional test-confirmed episodes. mRDT-defined infection incidence rates during the 0-4 month active detection period ranged across sites from 0.5 to 7.8, 0.3 to 8.4 and 0.3 to 3.3 episodes per person-year at risk among participants aged 5-11, 12-17 and 18-60 years, respectively. We will present age- and site-specific incidences, sensitivity and specificity of mRDT and TBS compared with qPCR, agreement between mRDT and TBS, and progression from submicroscopic (qPCR-positive/mRDT-negative) to mRDT-detectable infection. The MAINZ cohort study generated high-resolution, site-specific estimates of infection burden in older children, adolescents and adults, and provides critical inputs for the design and powering of *P. falciparum* infection-prevention vaccine trials in endemic African countries.

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POSITIVITY RATES AND PREDICTORS OF *PLASMODIUM FALCIPARUM* INFECTIONS IN CHILDREN UNDER 15 YEARS: A LONGITUDINAL HEALTH FACILITY-BASED SURVEY

Angelina J. Kisambale[1], Dativa Pereus[1], Rashid Ally Madebe[2], Salehe Mandai[1], Hussein Juma Semboja[1], Happyness A. Mwailunga[1], Laurencia S. Tangawizi[1], Rule Budodo[1], Gideoni Isaya Maulidi[1], Ninja Sugwejo Bisaga[1], Baraka W. Mbuba[3], Catherine Bakari[1], Gervas Chacha[2], Solomoni Mwakasungula[1], Daniel Protasy Challe[4], Daniel Anderson Petro[5], Doris David Mbata[2], Daniel Baraka Mbwambo[6], Sijenunu Aaron[7], Abdallah Suleiman Lusasi[7], Samuel Lazaro[7], Stella Kajange[8], Celine Isaack Mandara[2], Misago Dimson Seth[2], Deusdedith R.S. Ishengoma[1]

[1]Ifakara Health Institute, Dar Es Salaam, United Republic of Tanzania, [2]National Institute for Medical Research, Dar Es Salaam, United Republic of Tanzania, [3]Nelson Mandela African Institution of Science and Technology, Arusha, United Republic of Tanzania, [4]National Institute for Medical Research, Tanga, United Republic of Tanzania, [5]University of Dar es Salaam, Dar Es Salaam, United Republic of Tanzania, [6]National Malaria Control Program-Tanzania, Dodoma, United Republic of Tanzania, [7]National Malaria Control Programme, Dodoma, United Republic of Tanzania, [8]Prime Minister's Office, Regional Administration and Local Government, Dodoma, United Republic of Tanzania

Malaria, mainly caused by *Plasmodium falciparum,* remains a major public health burden in sub-Saharan Africa, and control interventions have traditionally focused on under-fives as the most vulnerable group. Recent evidence suggests that older children also carry a high burden of malaria and contribute to sustained transmission. This study assessed the prevalence and factors associated with *P. falciparum* infections among children aged ≤15 years from selected regions of Tanzania. The study used data from 5320 samples collected through a longitudinal health facility-based survey, initiated in 2022, covering five districts in five regions (Kagera, Kigoma, Njombe, Ruvuma and Tanga) of Mainland Tanzania. The samples were analysed using quantitative polymerase chain reaction (qPCR). Data analyses included estimation of positivity rates and determining factors associated with *P. falciparum* infections using multivariable analysis. The positivity rate by qPCR was 80.9%, and it was significantly lower (78.2%) among under-fives, compared to young children (aged 5 - <10 years, 86.0%; p < 0.001), and older children (10 - ≤15 years, 90.4%; p < 0.001). Children aged 5 - <10 years (adjusted odds ratios [aORs] = 1.70, 95% confidence interval [CI]: 1.43 – 2.02, p < 0.001) and those aged 10 - ≤15 years (aORs = 1.41, 95% CI: 1.13–1.76, p = 0.002) had higher odds of infections compared to under-fives. Compared to the reference region (Njombe), the odds of infections were significantly lower in Kigoma (aOR = 0.26, 95% CI: 0.19–0.36, p < 0.001), Ruvuma (aOR = 0.65, 95% CI: 0.47–0.91, p = 0.011), and Tanga (aOR = 0.38, 95% CI: 0.27–0.53, p < 0.001), while no significant difference was observed in Kagera (p = 0.630). Sex was not significantly associated with *P. falciparum* infections (p = 0.352). These findings highlight significant geographical heterogeneity in malaria transmission and indicate a higher burden of malaria among school children compared to under-fives. Expanding malaria control interventions beyond under-fives may be critical for reducing transmission and achieving the national malaria elimination targets.

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EARLY MALARIA INFECTIONS IN INFANCY IN WESTERN KENYA: EVIDENCE FROM A LONGITUDINAL BIRTH COHORT IN A HOLOENDEMIC SETTING

Kristan Alexander Schneider[1], Clinton Odhiambo Onyango[2], Sharley A. Wasena[3], Shamim Wanjiku Osata[4], Qiuying Cheng[5], Samuel Anyona[6], Douglas Jay Perkins[5]

[1]UNM, Albuquerque, NM, United States, [2]Maseno University, Albuquerque, NM, United States, [3]Maseno University, Kisumu, Kenya, [4]University of New Mexico-Kenya Global Health Programs, Nairobi, Kenya, [5]University of New Mexico, Albuquerque, NM, United States, [6]Univ of New Mexico, Albuquerque, NM, United States

Infants in the first 3 to 6 months of life have historically been considered relatively protected from malaria infection. However, recent evidence suggests early-life infections are common in high-transmission (holoendemic) settings such as western Kenya. Understanding the timing and characteristics of first malaria infections is important for characterizing malaria risk in early childhood. As such, we conducted a longitudinal birth cohort study at Siaya County Referral Hospital in western Kenya to evaluate the risk of malaria infection in infancy. A total of 570 children born to HIV-negative mothers were enrolled at birth and followed for 36 months with monthly and acute visits. After excluding children without a visit or with only one recorded visit within the first 180 days of life, 461 children were retained for data collection and analysis. The date of the first malaria episode (any *Plasmodium* species) diagnosed by microscopy and/or rapid diagnostic test (RDT) was recorded. The timing of the first infection was analyzed using a parametric survival model assuming a generalized gamma-distributed time-to-event. Clinical presentation and parasite density were compared between infections occurring in children <6 months and ≥6 months. Results revealed that 10% of children experienced a first malaria episode by day 107.8 (95% CI: 98.2-118.6), 20% children by day 150.4 (95% CI: 136.6-164.5), and over 50% of children within the first year of life (median=319.1 days, 95% CI: 287.2-353.3). Clinical symptoms did not differ between infections occurring before and after 6 months of age. However, parasite density differed significantly, with lower trophozoite counts in infections occurring in children <6 months (Mann-Whitney U test, p=0.0029), suggesting distinct parasitological characteristics of malaria in early infancy. Since malaria during early infancy is common in high-transmission settings, persistent *Pf*HRP2 antigenemia could lead to false-positive malaria rapid diagnostic test (RDT) results. Our findings show that malaria infection is common in infancy, with important implications for diagnosis and early risk stratification.

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INCREASING MALARIA BURDEN IN KINSHASA PROVINCE, DEMOCRATIC REPUBLIC OF THE CONGO (DRC): EVIDENCE FROM A LONGITUDINAL COHORT STUDY

Melchior Mwandagalirwa Kashamuka[1], Kristin Banek[2], Ruthly Z. Francois[3], Jack Kokolomami[4], Fabien Z. Vulu[5], Joseph L. Atibu[4], Nono M. Mvuama[4], Georges M. Emo[4], Joseph A. Bala[4], Tommy M. Nseka[6], Baudouin B. Matela[6], Georges Kihuma[4], Marthe Nkalani[4], Patou Wibina[4], Heritier M. Kimonekene[4], Samuel J. White[3], Rachel Sendor[7], Faustin Manenga[4], Thierry L. Bobanga[5], Varun Goel[8], Rhoel R. Dinglasan[9], Michael Emch[10], Victor Mwapasa[1], Jonathan J. Juliano[3], Jonathan B. Parr[3], Antoinette K. Tshefu[4]

[1]Kamuzu University of Health Sciences, Blantyre, Malawi, [2]Celia Scott Weatherhead School of Public Health and Tropical Medicine - Department of Epidemiology, Tulane University, New Orleans, LA, United States, [3]Institute of Global Health and Infectious Diseases, University of North Carolina, Chapel Hill, NC, United States, [4]Kinshasa School of Public Health, University of Kinshasa, Kinshasa, Democratic Republic of the Congo, [5]Department of Tropical Medicine, University of Kinshasa, Kinshasa, Democratic Republic of the Congo, [6]National Malaria Control Program, Kinshasa, Democratic Republic of the Congo, [7]Department of Epidemiology, Gillings School of Global Public Health, University of North Carolina, Chapell Hill, NC, United States, [8]Geography College of Arts and Sciences, University of South Carolina, Columbia, SC, United States, [9]Department of Infectious Diseases & Immunology, College of Veterinary Medicine, University of Florida, Gainesville, FL, United States, [10]Department of Geography, University of North Carolina, Chapel Hill, NC, United States

Malaria remains a major public health problem in the Democratic Republic of the Congo (DRC), yet data to guide control efforts are limited to infrequent household surveys and incomplete surveillance. Here, we estimate *Plasmodium falciparum* infection burden and factors associated over time. We conducted a prospective cohort study (2018–2022) across seven sites in urban, peri-urban, and rural areas of Kinshasa Province, with data from household and clinic visits. *P. falciparum* was detected by rapid

diagnostic test and qPCR. Temporal trends in prevalence were estimated using generalized estimating equations (GEE), cumulative incidence by Kaplan-Meier, and incidence rates using person-time at risk. GEE was used to identify correlates of infection. Among 1,635 participants, the overall *P. falciparum* burden was high; 45.1% and 75.7% of samples were qPCR-positive during household and clinic visits, respectively. We observed marked spatial heterogeneity of *P. falciparum* infection, highest in rural and peri-urban areas and lowest in urban areas. The average annual prevalence significantly increased by 2.8% (95% CI: 2.1% - 3.6%) over the study period. The one-year cumulative incidence of *P. falciparum* was 64.7%, rising to 98.6% by year four, with an overall incidence rate of 169 episodes per 100 person-years. Infection was associated with younger age, lower household wealth, and non-urban residence. These estimates exceed routine health system data (DHIS2). High prevalence and incidence indicate sustained and increasing malaria transmission in the region. Current surveillance methods underestimate the malaria burden across age groups and settings; adapted surveillance tools capturing multiple age groups integrated within surveillance and national surveys are needed. Sustained and innovative approaches are needed to address the heavy malaria burden in Kinshasa and throughout the DRC.

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EXTREMELY HIGH PREVALENCE OF SUBCLINICAL *PLASMODIUM MALARIAE* AND *P. OVALE* INFECTIONS IN WESTERN KENYA, WITH HIGH-DENSITY INFECTIONS OCCURRING ALMOST EXCLUSIVELY AMONG MODERATE-TO-HIGH-DENSITY *P. FALCIPARUM* CARRIERS

Henry Kamugisha[1], Colins Oduma[2], Delis Mattei-Lopez[1], Eric Ochomo[3], Cristian Koepfli[1]

[1]University of Notre Dame, Notre Dame, IN, United States, [2]Uzima University, Nairobi, Kenya, [3]Kenya Medical Research Institute, Kisumu, Kenya

The importance of *Plasmodium malariae* (Pm), *P. ovale wallikeri* (Pow), *P. ovale curtisi* (Poc), and *P. vivax* (Pv) in Africa is increasingly recognized. Data on their prevalence, transmissibility, and interactions with *P. falciparum* (Pf) are scarce. We developed a new qPCR assay that can detect all non-falciparum Plasmodium species, and new species-specific qPCR assays. We screened 3061 blood samples from subclinical individuals in western Kenya where prevalence of Pf was 49.8% by qPCR. 34.4% (1053/3061) of individuals carried non-falciparum infections, of which a species could be determined in 686 individuals. We observed a prevalence of 23.1% (708/3061) for Pm, 5.1% (156/3061) for Poc, 3.4% (103/3061) for Pow, and no Pv. 74.5% of non-falciparum infections were coinfections with Pf. Pronounced differences in parasite densities were observed between single- and multi-species infections. Many individuals carried moderate-to-high-density infections of multiple species. 85.4% (111/130) of P. malariae infections >100 parasites/µL were coinfected with *P. falciparum* also at densities >100 parasites/µL. Likewise, higher-density Poc and Pow infections were seen almost exclusively in individuals carrying *P. falciparum* infections >100 parasites/µL. In contrast, for both Pm and Pf, a strong reduction in the number of low-density infections (<10 parasites/µL) in mixed-species infections was observed. Pf gametocyte densities were not affected by co-infections. 73.0% (54/74) of Pm infections >10 parasites/µL harbored gametocytes, and densities were closely correlated with parasite density. In conclusion, we report among the highest prevalence of Pm and Po from Africa to-date. Non-falciparum infections reached highest densities in co-infections with Pf, pointing to a possible immune evasion mechanism. This finding contradicts the notion that Pm and Po transmission will increase if Pf prevalence decreases.

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IMPACT OF DISCONTINUING SEASONAL MALARIA CHEMOPREVENTION ON MALARIA INCIDENCE IN NORTHERN MOZAMBIQUE: A RETROSPECTIVE ANALYSIS (2023-2025)

Ivan Alejandro Pulido Tarquino[1], Sonia Maria Mauricio Enosse[1], Crispim Munda[2], Ebenezer Ikechukwu[3], Christian Rassi[4], Chuks Nnaji[4]

[1]Malaria Consortium, Maputo, Mozambique, [2]Malaria Consortium, Nampula, Mozambique, [3]Malaria Consortium, Abuja, Nigeria, [4]Malaria Consortium, London, United Kingdom

Seasonal malaria chemoprevention (SMC) has demonstrated substantial reductions in malaria incidence in Mozambique and across Africa. In Nampula Province, northern Mozambique, SMC was scaled up province-wide in 2022-2023, targeting around 1.5 million children aged 3-59 months during the peak malaria transmission season. In 2024-2025, SMC was discontinued due to operational constraints. We conducted a retrospective observational analysis using monthly district-level surveillance data from 2023 to 2025 to assess changes in malaria burden following withdrawal. Primary outcome was rapid diagnostic test (RDT) confirmed malaria among children > five years(U5). Exposure was defined at the seasonal level (SMC or non-SMC) and annual level (years with or without SMC). Descriptive analyses examined trends in outpatient consultations (OPD), confirmed cases, and test positivity rates (TPR). Poisson regression models with district-level clustered robust standard errors were fitted to estimate incidence rate ratios (IRRs), including an offset for population size and interaction terms. Across 2023-2025, 3,083,232 RDT-confirmed malaria cases were reported among U5 children out of 9,991,235 OPD consultations. Mean monthly incidence increased from 57.1/1,000 in 2022-23 to 66.5/1,000 in 2023-24 and 80.3/1,000 in 2024-25. Incidence was higher in 2023-24 (adjusted IRR 1.16, 95% CI 1.07-1.25, p<0.001) and substantially higher in 2024-25 (IRR 1.39, 95% CI 1.23-1.56, p<0.001). Test positivity rates showed a stepwise increase across years, with significant inter-annual differences (p<0.001). District-level analyses revealed marked heterogeneity in transmission intensity and widening dispersion in TPR in 2024-25. Sensitivity analyses using OPD consultations as exposure and alternative seasonal classifications yielded consistent patterns. The observed temporal patterns are indicative of a loss of the protective effect previously conferred by SMC. These findings support the role of SMC as a key contributor to malaria control in this high-transmission setting and the potential public health implications of halting delivery.

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ON THE LAST MILE OF MALARIA ELIMINATION IN COSTA RICA: MOBILE POPULATIONS AS THE DRIVER OF RESIDUAL TRANSMISSION, 2020-2025

Jennyffer Gonzalez[1], Sara Arce[2], Yadel Centeno[3], Rodrigo Marin[1], Isaac Vargas[4], Emma Margarita Iriarte[5], Leopoldo Villegas[6]

[1]Ministry of Health, San Jose, Costa Rica, [2]Consultor IREM, San Jose, Costa Rica, [3]Consultant IREM, San Jose, Costa Rica, [4]Ministry of health, San Jose, Costa Rica, [5]Inter American Development Bank-IDB, Washington, DC, United States, [6]International Advisor Independent, Silver Spring, MD, United States

As countries approach malaria elimination, transmission becomes increasingly concentrated in mobile and high-risk populations. Costa Rica illustrates this transition from generalized to mobility-driven transmission. A retrospective analysis of national surveillance data from 2020 to 2025 characterized malaria cases by demographic, occupational, geographic, and mobility-related factors. During the study period, malaria cases were highly concentrated among adult males (74%) and migrant populations (92%), indicating a marked shift toward transmission in socially and geographically mobile groups. Occupational exposure was a major driver, with miners accounting for 63% of all cases, reflecting the intense risk associated with specific economic activities linked to cross-border movement. Transmission was strongly associated with mobility across the

Costa Rica-Nicaragua border, with 40% of cases linked to exposure in Nicaragua. At the same time, persistent focal transmission was observed in mining areas such as Las Crucitas, where mobility and occupational risk converge. These findings indicate that imported infections and high-risk occupational settings jointly sustain residual transmission. Following the implementation of targeted interventions—including active surveillance, community-based screening, and the deployment of health inspectors in high-risk areas—malaria cases declined sharply from 520 in 2023 to 21 in 2025, a 96% reduction. This decline occurred despite continued population movement across border regions, suggesting that interventions tailored to mobile populations can effectively interrupt transmission. Malaria persistence in Costa Rica is no longer driven by widespread transmission but by importation and high-risk mobile populations. Targeted strategies addressing cross-border mobility, occupational exposure, and rapid case detection are essential to achieve and sustain malaria elimination in this and similar settings.

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PREDICTING EARLY MALARIA RECURRENCES IN VENEZUELA THROUGH CLINICAL AND EPIDEMIOLOGIC PATTERNS IN A VENEZUELAN COHORT

Oriana A. Regalado-Gutiérrez[1], David Alberto Forero-Peña[2], Fhabián S. Carrión-Nessi[3], Giancarlo Turri[1], Daniel Patiño[1], Óscar Noya-Gonzalez[1], Jaime Torres[1]

[1]Central University of Venezuela, Caracas, Bolivarian Republic of Venezuela, [2]Central University of Venezuela, Brimfield, MA, United States, [3]Navarra Center for International Development, Pamplona, Spain

Malaria recurrence remains a major challenge in endemic settings. In contexts such as Venezuela, where molecular surveillance is not routinely available, distinguishing relapse from recrudescence may rely on clinical and epidemiological patterns. We retrospectively analyzed confirmed malaria episodes diagnosed from 2017-2025 in a Venezuelan cohort to identify predictors of early malaria recurrence. Episodes were classified as primary infection, early recurrence, or late recurrence. Early recurrences were defined as episodes occurring within 42 days of the previous episode, while late recurrences occurred thereafter. Multivariable logistic regression with backward elimination (Wald) was used to identify independent predictors of early recurrence. A total of 5,615 malaria episodes were analyzed, including primary infections (n=2,026), early recurrences (n=927), and late recurrences (n=2,662). Median patient age was 30 years (IQR 23-41), and 58.5% were male. *Plamodium vivax* accounted for 83.0% of cases. Severe malaria occurred in 12.5% of episodes, and 40.1% of patients had incomplete previous treatment. In the adjusted model (Hosmer-Lemeshow p=0.052; Nagelkerke R^2=0.096), severe malaria (aOR 3.99, 95% CI 2.47-6.45; p<0.001), incomplete treatment (aOR 1.96, 95% CI 1.39-2.76; p<0.001), and higher parasite density (p=0.027) were independently associated with early recurrence. Early malaria recurrences in Venezuelan patients were strongly associated with severe disease, incomplete treatment, and parasite burden. Predictive models using routine clinical and epidemiological data may support risk stratification and targeted follow-up in settings with limited access to molecular surveillance.

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EXPLORING GENETIC VARIATION AND SELECTION SIGNATURES OF INSECTICIDE RESISTANCE IN *ANOPHELES GAMBIAE SENSU LATO* POPULATIONS IN GHANA

Anisa Abdulai[1], Sophie Moss[2], Kai Brandt-Templeton[3], Emma Collins[4], Isaac Kwame Sraku[1], Puck Van der Laan[3], Christopher Mfum Owusu-Asenso[5], Yaw Akuamoah-Boateng[1], Abdul Rahim Mohammed Sabtiu[1], Fred Aboagye-Antwi[1], Patrick Fred Ayeh-Kumi[1], Susana Campino[3], Yaw Asare Afrane[1]

[1]university Of Ghana, Accra, Ghana, [2]London School of Hygiene & Tropical Medicine, London, United Kingdom, [3]London School of Hygiene and Tropical Medicine, London, United Kingdom, [4]LSHTM, LONDON, United Kingdom, [5]university Of Ghana, Accra, AA, Ghana

Malaria remains a major public health burden in Sub-Saharan Africa, with Ghana contributing substantially to global cases and deaths. Insecticide resistance in malaria vectors threatens control efforts, yet integrated evidence linking phenotypic resistance with genomic variation across ecological and intervention settings remains limited. This study combines low-cost high-throughput amplicon sequencing and whole-genome analysis to characterize insecticide resistance patterns, genetic diversity, and selection signatures in *Anopheles gambiae* senso latu across diverse ecological zones and vector intervention settings in Ghana. WHO bioassays revealed moderate-to-high pyrethroid resistance (4-65% mortality) across all sites, with elevated organophosphate resistance in indoor residual spraying areas. Amplicon sequencing of 330 mosquitoes from 17 sites identified 31 non-synonymous mutations in key resistance genes (*vgsc*, *ace1*, *rdl*, *gste2*), including 13 known variants (L1014F, L1014S, N1570Y, V402L, I1527T, F1529C, A1746S, A296G, A296S, G280S, I114T, F120L, L119V). We detected 17 novel mutations, with T154S (24%) and A1553T (3%) above 1% frequencies. Population genetic analyses based on nuclear and mitochondrial markers showed low nucleotide diversity and limited structure, indicating high gene flow. Whole-genome sequencing of 32 mosquitoes from four ecologically distinct sites confirmed low diversity (π=0.003) and negative Tajima's D, consistent with recent selective sweeps or population expansion. Genome-wide scans identified strong positive selection in insecticide resistance genes, including cytochrome P450s, ion channels, and ABC transporters, particularly in pyrethroid-resistant populations. Despite weak population structure, localized divergence was observed in an island site without any vector intervention. These findings suggests that resistance is driven by multiple genetic mechanisms under strong selection pressure, shaped by intervention intensity and ecological context. This data will help develop tailored vector control strategies for malaria control in Ghana.

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DENSITY-DEPENDENT AMPLIFICATION OF DELAYED INSECTICIDE FITNESS COSTS BY THE MICROSPORIDIA MB SYMBIONT IN MALARIA VECTORS

Khadija Nyendesi Juma[1], Steeven Belvinos[2], Soromane Camara[3], Mwatum Maloba[2], Stancy Mandere Omboye[2], Collins Odongo[2], Polycarp Okumu Ogonji[2], Syprine Amolo Odera[2], Victor Atunga Mobegi[1], Jeremy Keith Herren1[2], Priscille Barreaux1[2]

[1]University of Nairobi, NAIROBI, Kenya, [2]International Centre of Insect Physiology and Ecology, NAIROBI, Kenya, [3]Institute Pierre Richet, research centre of the National Institute of Public Health, Bouake, Côte D'Ivoire

Insecticide resistance in *Anopheles* vectors jeopardizes malaria elimination. While the inherited endosymbiont Microsporidia MB provides a biological barrier to *Plasmodium* transmission, its interaction with conventional chemical control measures remains poorly characterized. Integrating behavioral cup assays (n = 2,114 Anopheles arabiensis from Ahero, Kenya, with moderate resistance), qPCR profiling, and mathematical modeling, we demonstrate that Microsporidia MB density dependently amplifies the delayed fitness costs of permethrin exposure. High symbiont densities required for effective malaria blocking (density > 1.0) simultaneously

maximize host vulnerability to insecticide exposure. Although offspring with maternally inherited infection (MB+) exhibit significantly lower immediate 1-h knockdown (54.5% vs 70.8%, $p < 0.001$) and 24-h mortality (48.1% vs 57.0%, $p = 0.01$) following Olyset® net exposure, this initial protection is superseded by a survival collapse. Lifespan decreased by 1.55 days per log10 increase in MB density ($p = 0.002$), resulting in a predicted > 99.9% reduction in malaria transmission potential specifically among the high-density vectors of epidemiological interest. Furthermore, MB+ status reduced blood-feeding success by 7% reduction under insecticide-treated conditions ($p = 0.003$). These findings reveal a targeted biological-chemical synergy that selectively compromises the most effective transmission-blocking vectors, providing a robust Integrated Vector Management (IVM) framework to suppress resistant populations.

6901

GENERATIONAL SELECTION, TRANSCRIPTOMICS AND FUNCTIONAL CHARACTERISATION REVEAL THE IMPACT OF ENVIRONMENTAL POLLUTANTS ON THE EVOLUTION OF INSECTICIDE RESISTANCE IN MALARIA VECTORS

Abdullahi Muhammad

Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Insecticide resistance is threatening malaria control. While the evolution and spread of resistance has been linked to scale-up in the distribution of public health insecticides, the role of environmental pollutants such as the polyaromatic hydrocarbons (PAHs) from industrial and agricultural use remains largely uncharacterized. The PAHs are potent ligands of the aryl hydrocarbon receptor (Ahr) transcription factors involved in the regulation of xenobiotic metabolizing enzymes, and potentially involved in insecticide resistance. Here, using field insecticide-resistant (Auyo) *An. coluzzii* and a laboratory-susceptible colony (Ngousso), we conducted a multi-generational selection experiment using naphthalene, fluorene and a mixture of both PAHs. After ten generations, the changes in susceptibility to insecticides were monitored using WHO bioassays and whole-transcriptome analysis (RNASeq) was conducted. Compared with the non-selected colony lines, PAH exposures significantly reduced pyrethroid and DDT resistance in the field population, suggesting fitness cost associated with established resistance. In contrast, Ngousso showed a significant increase in DDT resistance ($p = 0.01$) at the tenth generation. A significant increase in permethrin resistance was also observed at the seventh generation ($p = 0.03$). Several candidate genes from the major detoxification classes were overexpressed in the selected lines (including GSTe2, CYP6Z1, and CYP6P4); the most consistent with overexpression in all the selected lines were CYP6M4 and CYP4C27, as well as those from the Ahr pathway. Recombinant heterologous expression of *CYP6M4* coupled with HPLC metabolism assays revealed its ability to metabolise pyrethroids, including permethrin, deltamethrin, and α-cypermethrin, as well as PAHs (naphthalene and fluorene). These findings establish the role of environmental pollutants as additional drivers of metabolic insecticide resistance in *An, coluzzii.*

6902

CONTRIBUTION OF MICROBIAL LARVICIDING TO THE MANAGEMENT OF INSECTICIDE RESISTANCE IN A HIGHLY PYRETHROID RESISTANT POPULATION OF *ANOPHELES COLUZZII*

Diane Leslie Nkahe[1], Christophe Antonio-Nkondjio[2]

[1]University of Yaoundé I/OCEAC, Yaoundé, Cameroon, [2]OCEAC, Yaoundé, Cameroon

Larviciding using microbial compound is considered appropriate for controlling outdoor biting mosquitoes and for managing insecticide resistance. However, the influence of larviciding in managing pyrethroid resistance remains unexplored. This study investigated whether larviciding using the microbial-based larvicide VectoMax G is contributing to increase the susceptibility of *Anopheles coluzzii* populations to pyrethroids. Therefore, the susceptibility status of *An. coluzzii* field population was evaluated at different time points, before and during larviciding treatments. Simultaneously, *An. coluzzii* larvae were collected and spliced into four groups, each subjected to 21 generations of selection, as follows; (i):deltamethrin0.05%_only, (ii):vectomax_only, (iii):vectomax+deltame thrin0.05%, (iv):vectomax+deltamethrin0.05%+susceptible. Life-traits parameters was measured in the progeny. Kdr frequency, then expression profile of detoxification and oxidative genes was checked using qPCR. Phenotypic and genotypic parameters was compared between the first and the last generation of each laboratory colony and between field samples collected before and during larviciding, with "Ngousso" strain as control. First findings shown a decrease in resistance intensity to deltamethrin from high to low in field populations. Overall, the colony vectomax+deltametri n+susceptible displayed a fitness profile similar to the susceptible colony with short development time and long adult survival rate. Moreover, the kdr gene frequency was found to vary in the field and in the laboratory populations and a significant decrease in the overexpression profile of detoxification genes was detected in field population after larviciding implementation. Overall, this study suggests that, under larvicide treatment, resistance reversal is possible, but it may take longer to establish in a highly resistant population, subject to continuous insecticide selection pressure. Furthermore, the addition of new tools specifically targeting the larval stages could improve malaria vector control and insecticide resistance management.

6903

SUSTAINED EFFICACY OF CHLORFENAPYR-BASED NETS AGAINST *ANOPHELES FUNESTUS* CONTRASTS WITH REDUCED PERFORMANCE AGAINST SOME *ANOPHELES GAMBIAE* POPULATIONS IN CAMEROON: IMPLICATIONS FOR RESISTANCE MANAGEMENT

Brice Natchema Soh Fonkou[1], Bebert Fotso[1], Emilie Ngongang-Yipmo[1], Vanessa Ngannang[1], Wilfried Toutcha[1], Magellan Tchouakui[1], Charles S. Wondji[2]

[1]Centre for Research in Infectious Diseases, Yaoundé, Cameroon, [2]Liverpool Sch of Tropical Medicine, Liverpool, United Kingdom

Pyrethroid-only ITNs have lost efficacy in sub-Saharan Africa due to widespread resistance. Dual-ingredient chlorfenapyr (CFP) nets offer an alternative, but emerging reduced susceptibility in some populations could threaten their long-term efficacy. This study assesses CFP susceptibility, cross-resistance, and CFP-based net performance in *Anopheles gambiae* and *An. funestus* populations from Cameroon. *Anopheles funestus* s.l. were collected from Elende and Mibellon, and *An. gambiae* s.l. from Mangoum and Nkolondom. Susceptibility to pyrethroids (PYR) and CFP was assessed using standard WHO tube and bottle assays, while net efficacy was assessed in tunnel tests with a PYR-only net (Interceptor®), two dual-AI nets (Interceptor® G2, PermaNet® Dual), and a CFP-only net (CFP-100). Molecular assays were used to genotype key PYR-resistance markers, and qRT-PCR quantified gene expression to explore potential mechanisms of cross-resistance to CFP. Both species displayed high-intensity PYR resistance which was more pronounced in *An. gambiae.* However, *An. funestus* populations remained fully susceptible to CFP, and all CFP-based nets induced ≥80% mortality, indicating sustained bioactivation and strong efficacy against this species. In contrast, *An. gambiae* populations showed reduced CFP susceptibility and low CFP-net mortality (24–46%), suggesting early, stable tolerance. Survivors exhibited overexpression of *CYP6P5*, *CYP9K1*, and *CYP325A* in *An. funestus*, and *CYP6P3* and *CYP6M2* in *An. gambiae*. No positive cross-resistance between PYR markers and CFP was observed; instead, negative cross-resistance was evident, particularly in *An. gambiae* s.s. carrying combined *Kdr* L995F–*CYP6P3* E205D genotypes. Chlorfenapyr-based nets remain highly effective against *An. funestus* but show compromised performance against *An. gambiae* in some areas with emerging tolerance. The contrasting responses, together with evidence of negative cross-resistance, underscore the urgent need for intensified surveillance, targeted deployment, and integrated resistance management strategies to preserve the long-term utility of CFP-based interventions.

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CHLORFENAPYR-BASED NET INTERCEPTOR G2 REDUCES INFECTION AND PYRETHROID RESISTANCE IN *ANOPHELES GAMBIAE* FROM CAMEROON AND UGANDA

Magellan Tchouakui[1], Nadia Mane[1], Merveille Awoufack[1], Bebert Fotso[1], Amanda Bastos[2], Jonathan Kayondo[3], Emmanuel emmanuel[1], Charles S. Wondji[4]

[1]Centre for Research in Infectious Diseases, Yaoundé, Cameroon, [2]University of Pretoria, Pretoria, South Africa, [3]Uganda Virus Research Institute, Entebbe, Uganda, [4]Liverpool Sch of Tropical Medicine, Liverpool, United Kingdom

Vector control has substantially reduced malaria in Africa, but intense pyrethroid resistance in *Anopheles* mosquitoes now threatens these gains. Dual activeingredient LLINs such as Interceptor G2 (IG2), which combine pyrethroids with chlorfenapyr, may turn this resistance into an "evolutionary trap" by exploiting the same metabolic pathways that both confer pyrethroid resistance and activate chlorfenapyr. Here, we ask whether largescale IG2 deployment in Cameroon and Uganda can (i) restore phenotypic susceptibility, (ii) reduce Plasmodium infection in vectors, and (iii) drive measurable shortterm evolutionary change in resistance markers. Household surveys and net inspections showed high IG2 and other LLIN ownership (>90%) but only moderate use (<60% slept under a net the previous night), indicating that low and heterogeneous usage is now a critical barrier to impact. WHO and CDC bioassays nevertheless showed significantly higher pyrethroid mortality in *An. gambiae* s.l. at all IG2 sites 2-3 years postdeployment, indicating partial restoration of susceptibility. *Plasmodium* infection rates in fieldcollected mosquitoes declined modestly, consistent with improved vector control. Molecular analyses revealed no significant change in L995Fkdr and E2056DCYP6P3 frequencies between pre and postIG2 periods, despite strong laboratory fitness costs, suggesting that shortterm evolutionary responses at these loci are subtle. In contrast, CYP6Z1 and CYP9K1 expression declined significantly after IG2 deployment but remained above susceptiblestrain levels, indicating a quantitative reduction rather than elimination of metabolic resistance. Surveys of farmers and pesticide sellers revealed extensive agricultural insecticide use, which may counteract IG2 driven selection against resistance. Overall, IG2 appears to reduce resistance intensity and infection risk without rapid replacement by alternative resistance mechanisms, but realizing its longterm benefits will require integrated management of insecticide use across publichealth and agricultural sectors.

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THE I236MCYP6P4 MUTATION CONFERS HIGH FITNESS COSTS AND INCREASE SUSCEPTIBILITY TO CHLORFENAPYRBASED NETS IN *ANOPHELES GAMBIAE* FROM UGANDA

Bebert Fotso[1], Merveille Awoufack[2], Nadia Mane[2], Ambrose ORUNI[2], Jonathan Kayondo[3], David P. Tchouassi[4], Armanda Bastos[1], Charles S. Wondji[5], Magellan Tchouakui[2]

[1]University of Pretoria, Pretoria, South Africa, [2]Centre for Research on Infectious Diseases (CRID), Yaoundé, Cameroon, [3]Uganda Virus Research Institute, Entebbe, Uganda, [4]International Centre of Insect Physiology & Ecology, Nairobi, Kenya, [5]Liverpool Sch of Tropical Medicine, Liverpool, United Kingdom

Metabolic resistance is now widespread in African malaria vectors and is often driven by overexpressed or mutated cytochrome P450s, yet the fitness costs associated with these mechanisms and their interaction with different classes of insecticide-treated nets (ITNs) remain poorly defined. Focusing on a key metabolic marker, the I236M-CYP6P4 mutation, we assessed its impact on long-lasting insecticidal net (LLIN) efficacy and on key life-history traits of *Anopheles gambiae* s.l. The I236M-CYP6P4 mutation was strongly associated with survival on pyrethroid-only LLINs (PermaNet 2.0), with homozygous resistant (RR) mosquitoes showing a >7-fold higher likelihood of survival than homozygous susceptible (SS) individuals (OR = 7.4, 95% CI 2.7-21.2, P = 0.001). A similarly strong association was detected for pyrethroid-PBO nets (PermaNet 3.0; OR = 9.0, 95% CI 3.5-21.5, P < 0.0001), indicating that I236M-CYP6P4 substantially compromises both standard and PBO LLINs. In contrast, the mutation showed a negative association with survival on chlorfenapyr-based LLINs, including PermaNet Dual (OR = 2.9, 95% CI 1.3-6.3, P = 0.007) and Interceptor G2 (OR = 1.5, 95% CI 0.6-3.7, P = 0.3), supporting the utility of chlorfenapyr nets against this highly pyrethroid-resistant population. Fitness assays using the Mayuge × Kisumu F2 strain revealed substantial life-history costs associated with I236M-CYP6P4, including reduced larval development and adult longevity, leading to a decline of the resistant allele over generations, despite a heterozygote advantage for fecundity and egg viability. By contrast, in a pure field-derived resistant Mayuge colony maintained for 14 generations without further selection, the marker remained fixed and resistance remained intense, whilst CYP6P4 expression declined over time, suggesting the involvement of compensatory mechanisms. These findings elucidate how fitness costs shape the reversal of insecticide resistance when applied early and provide actionable evidence to inform resistance management strategies, including mixture or rotation.

6906

COMPARATIVE TRANSCRIPTOMICS REVEAL CONSERVED CORE MECHANISMS OF TYPE I AND II PYRETHROID RESISTANCE ESCALATION IN *ANOPHELES FUNESTUS*

Ambrose Oruni[1], Vanessa Ngannang-Fezeu[1], Mahamat Gadji[1], Hervé Raoul TAZOKONG[2], Magellan Tchouakui[3], Mersimine F.M Kouamo[1], Jonathan Kayondo[4], Jack Hearn[5], Charles S. Wondji[6]

[1]Centre for Research in Infectious Diseases, Yaounde, Cameroon, [2]University of Yaoundé 1 and Centre for Research in Infectious Diseases, Yaoundé, CE, Cameroon, [3]Centre for Research in Infectious Diseases, Yaoundé, Cameroon, [4]Uganda Virus Research Institute, Entebbe, Uganda, [5]Centre of Epidemiology and Planetary Health, School of Veterinary Medicine, Scotland's Rural College, Inverness, United Kingdom, [6]Liverpool Sch of Tropical Medicine, Liverpool, United Kingdom

Escalating pyrethroid resistance in African malaria vectors threatens vector control tools. Differential pathways between type I and type II pyrethroids under increasing selection pressure remains largely unknown. Here, we present the first high-resolution comparative transcriptomic analysis of escalating permethrin (type I) and alpha-cypermethrin (type II) resistance using Anopheles funestus. RNAseq analysis revealed a conserved resistance framework and pathways of the core resistance genes dominated by overexpression of duplicated CYP6P9 and CYP6P4, CYP6P5, and CYP9K1 across both pyrethroid classes and resistance intensities, validated by qPCR and RNAi. Additional detoxification genes and pathways were mainly upregulated at escalated doses. SNP variant analysis identified non-synonymous variants in the key detoxification genes, as potential markers of resistance escalation. Genome selection scans showed no strong signals at known resistance loci except for regions around chromosomal inversions. The findings demonstrate that resistance to both pyrethroid classes and intensity is driven by shared, highly selected metabolic machinery, informing cross-resistance management and malaria vector control.

6907

INTEGRATED GENOMIC AND TRANSCRIPTOMIC ANALYSES REVEAL TARGET-SITE AND METABOLIC DRIVERS OF ORGANOPHOSPHATE RESISTANCE IN *ANOPHELES GAMBIAE*, A MAJOR MALARIA VECTOR IN CENTRAL AFRICA

Judith Labou Dandi[1], Francis Zeukeng[2], Arnaud Tepa[1], Vanessa Ngannang[1], Charles S. Wondji[3]

[1]Centre for Research in Infectious Diseases, Yaoundé, Cameroon, [2]The Biotechnology Center, University of Yaoundé I, Yaoundé, Cameroon, [3]Liverpool Sch of Tropical Medicine, Liverpool, United Kingdom

Malaria control in sub-Saharan Africa mainly relies on insecticide-based interventions such as Long-Lasting Insecticidal Nets (LLINs) and Indoor Residual Spraying (IRS). However, resistance in major malaria vectors

threatens the effectiveness of these tools. To mitigate widespread pyrethroid resistance, the World Health Organization recommended organophosphates such as pirimiphos-methyl (PM) for IRS. Yet, in Central Africa—where IRS deployment has been limited—the molecular basis of resistance to these compounds remains poorly understood. Following phenotypic detection of PM resistance in *Anopheles gambiae* from Nkolondom (Cameroon), we implemented a dual high-throughput sequencing approach. Targeted adaptive sampling using Oxford Nanopore long-read sequencing was applied to a 5Mb region of chromosome 2R in 10 resistant (alive) and 10 susceptible (dead) mosquitoes to characterize structural variants and target-site mutations. In parallel, Illumina NovaSeq RNA sequencing was performed to investigate metabolic resistance across exposed, unexposed field populations, and the Kisumu laboratory strain. This integrative multi-omics approach revealed complementary resistance mechanisms. Long-read sequencing showed a strong enrichment of the ACE-1 G280S resistance allele in alive mosquitoes (mean frequency: 87%), with all individuals carrying at least 70% of the resistant allele across copies. In contrast, dead mosquitoes exhibited a very low frequency (mean: 8%), with most individuals lacking the resistance allele. A 203Kb tandem duplication—previously reported in West and East Africa—was consistently detected in resistant individuals, supporting for the first time its role in Central Africa. Transcriptomic analyses revealed a distinct metabolic profile marked by overexpression of digestive, sensory, and oxidative enzymes, and downregulation of specific cytochrome P450 genes, including CYP9M1. These findings highlight the combined contribution of target-site and metabolic processes in driving organophosphate resistance, providing critical insights to inform vector control strategies.

6908

SCREENING LATIN AMERICAN IMMIGRANTS FOR CHAGAS DISEASE AND CLIMATE DRIVERS OF MIGRATION: PREPARING FOR THE FUTURE OF VECTOR-BORNE DISEASES IN THE SETTING OF A WARMING WORLD

Evan Czulada[1], Esha Banwatt[2], Audra Bass[1], Sarah Johnson[1], Vyjayanti Kasinathan[1], Yun Wang[1], Jessica K. Fairley[1]

[1]Emory University, Atlanta, GA, United States, [2]UT Southwestern Medical School, Dallas, TX, United States

Chagas disease screening remains inadequate in many Latin American populations in the United States, leading to life-threatening heart and gastrointestinal disease when not caught early. At the same time, climate change is leading to a spread of the triatomine vector in endemic areas while also driving individuals, either directly or indirectly, to emigrate out of vulnerable areas of Latin America. In order to pilot a Chagas screening program and to better understand climate drivers of migration, we enrolled individuals who had immigrated from Latin America (ages 6 years and up) at Atlanta-area healthcare facilities in 2021 and 2023. We measured serologic markers of Chagas disease in two separate assays and collected survey data on motivations for migration, occupation, Chagas disease risk factors, living situations, and environmental changes in their country of origin. We then analyzed the data descriptively and through chi-square methods to identify associations among the different variables. Among 105 study participants, five tested positive on one Chagas test (including 3 on RDT) with one participant confirmed with Chagas disease on follow-up ELISA, giving a prevalence of 0.95%. The majority of participants were from Mexico and El Salvador with a wide range of time living in the US. Nineteen participants (18%) reported that at least one of their motivations for emigration was climatic or extreme weather change, such as flooding, hurricanes, drought, and water shortages. Those from Central America were more likely to both recognize the triatomine insect (OR 4.95, 95% CI 1.03, 25.32) as well as report a climate reason for migration (4.19, 95% CI 1.78, 10.37). While we identified only 1 case of Chagas disease, this prevalence is in line with other studies on other Latin American populations in the US. Importantly we found many participants who listed weather or climatic reasons to migrate and an overlap with those from Central America both identifying the triatomine bug and having a climate driver of migration. There is an urgent need to improve screening of Chagas disease in the US and better understand risk factors for Chagas in a changing climate.

6909

INTEGRATING KINSHIP NETWORKS INTO SPATIAL RISK PREDICTIONS OF DOMICILIARY VECTOR INFESTATION AND CHILD *TRYPANOSOMA CRUZI* INFECTION IN RURAL ARGENTINA

Katie Tseng[1], Maria Sol Gaspe[2], Maria del Pilar Fernández[1]

[1]Washington State University, Pullman, WA, United States, [2]University of Buenos Aires, Buenos Aires, Argentina

Transmission of vector-borne pathogens is often attributed to biological and environmental processes that shape ecological hazard. However, whether this hazard translates into actual infection risk depends on human behaviors that determine exposure to sources of infection. In the Argentine Chaco where Chagas disease is endemic, residential mobility (the frequent relocation and rebuilding of houses) has been associated with lower risk of human *Trypanosoma cruzi* infection, suggesting that mobility may disrupt persistent exposure to domiciliary vector infestation. At the same time, routine inter-household interactions embedded in social networks may also influence vector movement and exposure pathways, but these processes remain largely unexamined in vector-borne disease systems. In this study, we examined the role of social networks in shaping *T. cruzi* transmission by reconstructing the kinship network of 403 households across rural communities of Chaco Province, Argentina. We incorporated network measures into Bayesian spatial models of domiciliary infestation with *Triatoma infestans* and human *T. cruzi* infection, and used scenario-based prediction to assess how social connectivity and network accessibility interact with other eco-social drivers. We found that household mobility predicted lower risk of domiciliary infestation and child *T. cruzi* infection when households were socially isolated, but this protective effect was attenuated among socially connected households. Additionally, greater social accessibility was associated with higher infection risk among children but only in the absence of domiciliary infected vectors. When domiciliary infected vectors were present, accessibility decreased infection risk. These findings demonstrate that the social network can modify risk in ways not captured by spatial proximity alone, particularly in interaction with residential mobility and infected vector abundance, thus warranting consideration in the design of Chagas disease control strategies, particularly among highly mobile populations.

6910

CHARACTERIZING ARRHYTHMIA RISK IN PEOPLE WITH CHAGAS DISEASE: A SINGLE CENTER EXPERIENCE IN BOSTON, MASSACHUSETTS

Briana Le[1], Katherine Anne Reifler[1], Jack McNamara Hensien[1], Natalia Diaz Clesse[2], Andrea Azocar[2], Alexander Schade[2], Madolyn Dauphinais[3], David H. Hamer[4], Deepa M. Gopal[1], Daniel L. Bourque[2]

[1]Boston University Chobanian and Avedisian Medical School, Boston, MA, United States, [2]Boston Medical Center, Boston, MA, United States, [3]University of Massachusetts Chan Medical School, Worcester, MA, United States, [4]Department of Global Health, Boston University School of Public Health, Cambridge, MA, United States

Chagas disease (CD) is associated with life-threatening arrhythmias, occasionally without antecedent symptoms. Risk factors associated with malignant arrhythmias remain poorly understood in CD. We conducted retrospective chart review comparing patients at Boston Medical Center diagnosed with CD from 2011-2026 with and without arrhythmia-related events, including malignant arrhythmias and/or cardiac device placement (implantable loop recorder [ILR], pacemaker, or implantable cardioverter-defibrillator [ICD]). Of 222 patients with CD, 12% (27) had an arrhythmia-related event including 21 (9%) with a malignant arrhythmia that required intervention, and 6 (3%) with device placement for primary prevention. Eighteen out of 27 (67%) had cardiac device placement, including ILR (3), pacemaker (4), and ICD (12); one transitioned from ILR to ICD. Compared to patients without arrhythmia-related events, a greater proportion of those with events were >50 years old (15/27, 56% vs 44/195, 23%), had

hypertension (19/27, 70% vs 61/195, 32%), hyperlipidemia (22/27, 81% vs 62/195, 32%), or coronary artery disease (11/27, 41% vs 8/195, 4%), and had elevated BNP (18/25, 72% vs 10/80, 13%); these differences were statistically significant (p<0.05). Three patients (11%) with arrhythmia-related events had normal electrocardiograms at the time of CD diagnosis, compared to 116/191 (61%) without an event (p<0.05). Apical hypokinesis was more common among patients with an event (10/26, 39%) compared to those without (9/172, 5%; p<0.05). Of the 35 patients with cardiac MRI, late gadolinium enhancement (LGE) was seen in 18 (51%). Half of the patients with LGE had an event (9/18) vs 1/14 (1%) of individuals without LGE had an event (p<0.05). These data suggest that comorbidities such as hypertension, hyperlipidemia and coronary artery disease may be associated with arrhythmia-related events in people with CD. A normal ECG at the time of CD diagnosis did not preclude arrhythmia-related events. Echocardiogram findings, including apical hypokinesis, and LGE enhancement may indicate increased risk of arrhythmia in patients with CD.

6911

EXPOSURE TO *TRYPANOSOMA CRUZI* IN RURAL COMMUNITIES OF THE BOLIVIAN CHACO

Dayana Nicole Serrano Olmos[1], Luciana Lidia Basma Melgar[1], Franco Ignacio Soto Mercado[1], Ana María Montaño Arias[2], Beatriz Amparo Rodriguez-Olguin[3], Brandon N Mercado-Saavedra[4]

[1]Instituto de Investigación de Medicina, Universidad Católica Boliviana San Pablo, Santa Cruz de la Sierra, Plurinational State of Bolivia, [2]Unidad Funcional de Entomología Médica, Centro Nacional de Enfermedades Tropicales, Santa Cruz de la Sierra, Plurinational State of Bolivia, [3]Facultad de Ciencias Farmacéuticas y Bioquímica, Universidad Autónoma Gabriel René Moreno, Santa Cruz de la Sierra, Plurinational State of Bolivia, [4]Instituto de Investigación de Medicina, Universidad Católica Boliviana San Pablo, Santa Cruz, Plurinational State of Bolivia

Chagas disease remains a major public health challenge in Latin America, where it persists as a neglected tropical disease. In Bolivia, it primarily affects rural populations, especially in endemic regions such as the Bolivian Chaco, where transmission dynamics are poorly characterized. To address this gap, we conducted a cross-sectional study in June 2025 in the communities of Palmarito and Choroketi, in eastern Bolivia, where active searches for triatomine bugs were performed inside and around dwellings. Collected vectors were analyzed for *Trypanosoma cruzi* infection and genetic variability (DTU) using PCR. Concurrently, the serological status of adults was assessed using a commercial ELISA, a rapid test, and a confirmatory indirect immunofluorescence assay (IFA), and demographic data were collected through structured questionnaires. Overall *T. cruzi* seroprevalence was 67% (87/130), indicating high exposure in both communities. Human infection was significantly associated with increasing age and number of children, with no differences by sex. Household environmental factors, such as the use of latrines and the presence of farm animals, were also significantly associated with infection. Most of the captured vectors were *Triatoma infestans*. A higher proportion of adult triatomines was found in Choroketi (79%), while nymphs were more frequent in Palmarito (59%), suggesting active domestic colonization. The overall *T. cruzi* infection rate in vectors was 18%, with a significantly higher proportion in Choroketi (50%) compared to Palmarito (13%). Furthermore, DTUs III and V were identified in both communities, confirming the co-circulation of these lineages in the domestic environment. Collectively, these findings demonstrate the presence of *T. cruzi* in triatomines collected within dwellings and suggest a domestic transmission risk environment, where the population remains continuously exposed to the parasite, compounded by the co-circulation of lineages III and V detected in this region of the Bolivian Chaco.

6912

HIGH PREVALENCE OF *TRYPANOSOMA CRUZI* INFECTION IN RACCOONS (*PROCYON LOTOR*) IN CENTRAL ILLINOIS, UNITED STATES

Ozioma Esther Onuselogu

Eastern Illinois University, Charleston, IL, United States

Chagas disease, caused by the protozoan parasite *Trypanosoma cruzi*, is an emerging zoonotic infection in the United States, yet the distribution and prevalence of infection among wildlife reservoirs remain incompletely characterized in many regions. Raccoons (*Procyon lotor*), which are widely distributed across rural, peri-urban, and urban environments, are recognized hosts of *T. cruzi* and may contribute to parasite persistence in areas where human exposure can occur. This study evaluated the prevalence of *T. cruzi* infection in raccoons from Central Illinois and compared regional infection patterns with previously reported prevalence estimates from other areas. Raccoon carcasses were obtained from roadkill and licensed trapper collections across rural and urban landscapes. Heart tissue samples from 78 raccoons collected in Central Illinois were screened for *T. cruzi* using molecular diagnostic methods. Infection prevalence in Central Illinois raccoons was 69.2% (95% CI: 58.94-79.46%), substantially higher than prevalence reported in raccoons from Southern Illinois (18.9%; 95% CI: 6.31-31.49%) and Louisiana (33.6%; 95% CI: 25.12-42.08%). Regional differences in infection prevalence were significant by Kruskal-Wallis analysis *($p < 0.001$)*, and chi-square testing confirmed a significant difference between Central Illinois and previously reported Illinois populations *($p < 0.0001$)*. These findings reveal marked regional variation in *T. cruzi* infection among raccoon populations and highlight the importance of wildlife surveillance for understanding transmission dynamics and informing risk assessment for Chagas disease.

6913

TIME FROM SYMPTOM ONSET TO TREATMENT INITIATION IN VISCERAL LEISHMANIASIS IN BRAZIL, 2007-2023: A NATIONAL REGISTRY-BASED STUDY

Juan Sebastian Hurtado Zapata[1], Prabin Dahal[2], James Patrick Wilson[3], Makoto Saito[4], Philippe J. Guerin[1], Glaucia Cota[5], Neal D. Alexander[6], Dawit Getachew Assefa[7]

[1]Infectious Diseases Data Observatory, Oxford, United Kingdom, [2]IDDO, University of Oxford, United Kingdom, [3]Infectious Diseases Data Observatory (University of Oxford), Oxford, United Kingdom, [4]University of Tokyo, Oxford, United Kingdom, [5]DNDi, Rio de Janeiro, Brazil, [6]Centro Internacional de Entrenamiento e Investigaciones Medicas - CIDEIM, Cali, Colombia, [7]Dilla, Ethiopia

Visceral leishmaniasis (VL) is a notifiable disease in Brazil requiring reporting to the national disease registry (SINAN). Prompt diagnosis and treatment have remained the cornerstone of VL control, but treatment delays have been reported. We analyzed individual patient records reported to the SINAN registry (2007-2023) to characterize the time from symptom onset to treatment initiation (TTI) among confirmed VL cases and assessed factors associated with log-transformed TTI. During the study period, 151,604 VL notifications were reported, of which 35,186 primary VL cases with adequate information available to calculate TTI were eligible for inclusion. The median TTI was 27 days (interquartile range [IQR]: 14-55). TTI increased with patients' age, from 19 days (IQR: 11-32) in infants <1 year to 35 days (IQR: 18-73) in patients aged 16-50 years, and 34 days (IQR: 17-72) in those >50 years. The TTI distribution was best described as log-normal, with the model indicating 50%, 75%, and 95% of patients initiating treatment by 26, 56, and 169 days, respectively. Longer TTI intervals were observed in males (geometric mean ratio [GMR], 95% confidence interval [CI]: 1·03, 95% CI 1·01-1·06, ref: females), and in patients from the South region (GMR: 1·45, 95% CI 1·16-1·82, ref: Northeast), while progressively shorter TTI intervals were observed with increasing calendar period (GMR for 2019-2023 period: 0·86, 95% CI 0·83-0·89, ref: 2010-2014). Increase in TTI interval was associated with elevated odds of oedema, jaundice, and haemorrhage at presentation. Follow-up treatment outcomes were available

in 31,177 patients. The Kaplan-Meier survivorship at 12-months was 0.86 (95% CI: 0.83-0.89) in those presenting with delays of ≥120 days compared to 0.89 (95% CI: 0.87-0.92) in those with delays of <14 days and 0.91 (95% CI: 0.90-0.92) in those with delays of 15-30 days (P ≤0·001, log-rank tests for both pairwise comparisons). These findings suggest that delays in treatment initiation are associated with poor prognosis in Brazil and support targeted interventions for patient groups at higher risk of delayed diagnosis and poor outcomes.

6914

PATIENTS WITH ORALLY ACQUIRED SYMPTOMATIC ACUTE CHAGAS DISEASE EXPERIENCE HIGH RATES OF TREATMENT FAILURE

Dilma de Souza[1], Ana Yece Neves Pinto[2], Tiago Franca[3], Rafael Soares[4], Marcus Vinicius Guimarães De Lacerda[5], Viviane Cardoso[1], Taynah Puty[6], Alan Diego de Moura Farias[2], Thais Oliveira[6], Vitorina Marques[6], Thiago Teixeira[4], Alan Rebelo[4], Maria Paula Mourão[5], Xiaojun Zhao[7], Jana Janulikova[8], Rana Said[9], Ki Milligan[10], Alejandro Gabriel Schijman[11], Arturo Muñoz-Calderon[11], Srinivasa P S Rao[12], Malgorzata Leyk[13], Karin Meiser[14], Megan Barton[9], Linda Armstrong[15], Joseph Sidoti[9], Ana Blotta Baptista[16], Marina Pontello[16], Barbara Cristina Alves Mellado[16], Daria Arustamanian[8], Kristen Stevenson[9], **Natasha Hochberg**[9]

[1]Hospital de Clínicas Gaspar Vianna, Belem, Brazil, [2]Instituto Evandro Chagas, Belem, Brazil, [3]Health & Care Consultoria, Pesquisa, Atendimento e Gestão de Saúde Ltda, Belem, Brazil, [4]Hospital Universitário – Universidade Federal do Amapá, Amapa, Brazil, [5]Fundação de Medicina Tropical, Manaus, Brazil, [6]Health & Care Consultoria, Pesquisa, Atendimento e Gestão de Saúde Ltda, Belem, Brazil, [7]Novartis Biomedical Research, Cambridge, MA, United States, [8]Novartis, Prague, Czech Republic, [9]Novartis, Biomedical Research, Cambridge, MA, United States, [10]Novartis, Bethesda, MD, United States, [11]CONICET-INGEBI, Buenos Aires, Argentina, [12]Novartis Institute for Tropical Diseases, Singapore, Singapore, [13]IQVIA, Durham, NC, United States, [14]Novartis, Biomedical Research, Basel, Switzerland, [15]Novartis Biomedical Research, East Hanover, NJ, United States, [16]Novartis, Sao Paolo, Brazil

Studies of patients with orally acquired symptomatic acute Chagas disease (sACD) are limited, and few data exist on treatment response. Patients ≥12 years of age with consistent symptoms and a diagnosis of sACD based on microscopy, polymerase chain reaction (qPCR) or serology (IgM and two IgG assays) were included in this non-interventional observational study. At five northern Brazilian centers, patients were treated with benznidazole (BZN) per local practice and followed for 6 months. The study assessed *Trypanosoma cruzi* discrete typing units (DTU) at baseline (or follow-up if baseline parasitemia was low) and qPCR serially over 6 months (using a single DNA extraction and two qPCR reactions per timepoint). Forty patients were enrolled (median age 32.5 years, 60% male) including one from Amazonia state and the rest from Para and Amapa; 35 completed the 6-month follow-up. The median reported duration of BZN treatment was 60 days. Of the 38 with DTU data, 24 (63%) were infected with single DTUs (n=12 Tc1, n=6 TcIII, n=4 TcIV, and n=2 TcVI), 10 (26%) had mixed infections (with combinations of TcI, TcII, TcIII and TCIV), and in 4 (11%) DTU was not identified due to low parasite load. Of the 35 (88%) patients who were qPCR positive at baseline, 34 had 6-month qPCR data and 18 (53%) were qPCR positive. Only 7 (21%) remained qPCR negative at all time points after treatment (at 2, 3, and 6 months). Median parasite load (satDNA copies in log 10 scale) decreased from 5.4 log 10 cp/mL (interquartile range [IQR] 3.1-6.0) at baseline to 2.1 log 10 cp/mL (IQR 2.1-2.9) at 2 months and 2.7 log 10 cp/mL (IQR 2.1-3.1) at 6 months. In this cohort of patients with sACD, the majority are qPCR positive after treatment despite taking the standard BZN regimen. These results suggest a need for novel therapies, new approaches to current therapies, and/or enhanced treatment adherence support. Additional analyses may explore the predictors and mechanisms of treatment failure. Furthermore, to our knowledge, this study represents the first report of TcIII in human oral sACD from northeastern Brazil outside the Amazon region; oral outbreaks have been mainly due to TcI or incompletely typed DTUs.

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REASSESSING CHAGAS TRANSMISSION RISK IN BELIZE THROUGH INTEGRATED HUMAN SEROLOGY AND VECTOR GENOMICS

Kathleen Marie Weimer[1], Sarah M. Gunter[2], Silvia Justi[3], Claudia P. Herrera[4], Russell Manzanero[5], Alexander Kneubehl[2], Shannon Ronca[2], Gerhaldine Morazan[5], Kristy Murray[6]

[1]Emory University, Atlanta, GA, United States, [2]Baylor College of Medicine, Houston, TX, United States, [3]Charles County Public Schools, La Plata, MD, United States, [4]Tulane University, New Orleans, LA, United States, [5]Belize Ministry of Health and Wellness, Belmopan, Belize, [6]Emory, Decatur, GA, United States

Chagas disease, caused by *Trypanosoma cruzi,* remains the leading cause of non-ischemic dilated cardiomyopathy in the Americas, impacting >6 million lives, yet transmission dynamics in Belize are poorly characterized. In 2012, Belize was certified as having interrupted vector-borne *T. cruzi* transmission. However, in 2020, detection of an acute pediatric case through national acute febrile illness (AFI) surveillance, along with discovery of a novel triatomine vector species around the case-patient's home, prompted reassessment of *T. cruzi* exposure and circulation. We examined: 1) *T. cruzi* IgG seroprevalence among AFI participants; 2) phylogenetic analysis of local triatomines; 3) discrete typing unit (DTU) characterization from humans and vectors; and 4) geospatial mapping of vector occurrence and human seropositivity. Screening a subset of AFI participants (n = 563) identified 4 IgG-positive individuals, including a relative of the pediatric case, yielding a seroprevalence of ~0.7%. Positives occurred in 2 districts, paralleling vector exposure reports: ~1% of all AFI participants noted triatomine bites within 2 weeks of illness onset, with higher exposure in northern Corozal (4%) than southern Punta Gorda (2%). Multilocus phylogenetic analysis of 5 *Triatoma* specimens showed 1 clustering with *T. dimidiata* s.l. and 4 near *T. huehuetenanguensis,* with genetic distances supporting inter and intraspecific relationships. *T. cruzi* infection was identified in 1 of the 4, whose COX1 divergence (~97% identity) and distinct morphology suggests a cryptic or potentially novel lineage. DTU typing identified TcIV, supported by 89,746 high-quality reads mapping exclusively to this lineage. Miniexon phylogenies recovered 4 unique TcIV haplotypes related to TcIV found in southern Belize vectors and the acute pediatric case, yet distinct from Brazilian reference strains. These findings challenge the assumption that Chagas transmission in Belize is fully interrupted and underscore the need for renewed surveillance integrating serology, vector phylogenetics, and parasite genotyping to clarify transmission risk locally and globally.

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RISK FACTORS FOR VERTICAL TRANSMISSION OF *TRYPANOSOMA CRUZI* IN AN URBAN AREA OF SANTA CRUZ, BOLIVIA

Ayla Bullock[1], Freddy Tinajeros[2], Paloma Samame[3], Emily Joany Arteaga carrasco[3], Jean karla Velarde[3], Clariza Roxana Chavez[3], Liseth Roque Velasquez[4], Brenda Vasquez Martinez[1], Oksana Kharabora[1], Maria de Carmen Menduina[5], Beth Jessy Condori[6], Monica Jehnny Pajuelo[6], Manuela Renee Verastegui[6], Maritza Calderon[6], Robert H. Gilman[7], **Natalie Bowman**[1]

[1]University of North Carolina at Chapel Hill, Chapel Hill, NC, United States, [2]Johns Hopkins Univ, Santa Cruz, Plurinational State of Bolivia, [3]ETTA Projects, Santa Cruz, Plurinational State of Bolivia, [4]ETTA Projects, Santa Cruz de la Sierra, Plurinational State of Bolivia, [5]Hospital de La Mujer Percy Bola,nd, Santa Cruz de La Sierra, United States, [6]Universidad Peruana Cayetano Heredia, Lima, Peru, [7]Johns Hopkins Univ Bloomberg School of Public Health, Baltimore, MD, United States

Congenital Chagas disease remains a public health problem in the Americas. Prior studies of congenital Chagas disease identified higher maternal parasite load and twin birth as risk factors for transmission. While results have not always been consistent study to study, literature suggests that immunological and/or parasitological factors may affect risk. Less is known about the effects of maternal and pregnancy-related

clinical factors on vertical transmission. To address these questions, we are conducting a prospective cohort study of *Trypanosoma cruzi*-infected pregnant women and their infants at Hospital de la Mujer Percy Boland, a tertiary women's hospital in Santa Cruz, Bolivia. Women are recruited at prenatal visits or delivery and screened for Chagas disease by rapid serological test, confirmed with HAI assay. Infants of seropositive women are tested for Chagas disease by qPCR and TESA at birth, 1 month, and ≥9 months of age and by IgG ELISA at ≥9 months. Clinical and laboratory data are measured by study staff and abstracted from the medical record. We have enrolled 257 seropositive women, and 16 pregnancies resulted in vertical transmission, including one set of twins who both had congenital Chagas disease (transmission rate 6.25%). One vertically infected infant died of complications of prematurity. Women who transmitted were 4 years younger than nontransmitters (p=0.06). Premature rupture of membranes occurred in 24% of transmitters but 3.5% of nontransmitters (p=0.0002). No other maternal clinical features were associated with vertical transmission of *T. cruzi*, including immunosuppression, comorbidities, nutritional status, preeclampsia, or twin birth. We plan to further examine these features in multivariate analyses with a more complete data set, as data collection is ongoing.

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BIOMARKER IDENTIFICATION AND SEROLOGICAL LATERAL FLOW ASSAY (LFA) DEVELOPMENT FOR SURVEILLANCE OF *SCHISTOSOMA HAEMATOBIUM*

Megan McLarty[1], Anthony Sacco[1], Noriko Kobayashi[2], Daniela Oltean[3], Jean Saunders[1], Matthias Schwarz[4], Harisha Ramachandraiah[1], Dziedzom Komi de Souza[5], Shinjiro Hamano[2], Marco Biamonte[1]

[1]Drugs & Diagnostics for Tropical Diseases (DDTD), San Diego, CA, United States, [2]Inst Trop Med, Nagasaki Univ, Nagasaki, Japan, [3]Big Eye Diagnostics, San Diego, CA, United States, [4]Drugs & Diagnostics for Tropical Diseases (DDTD), Eggersriet, Switzerland, [5]Noguchi Memorial Institute for Medical Research, Legon, Ghana

Schistosoma haematobium (*Sh*), the cause of urogenital schistosomiasis, accounts for nearly half of schistosomiasis cases and is linked to serious complications, including female genital schistosomiasis (FGS). The current diagnosis relies on microscopic detection of *Sh* eggs in filtered urine; however, this method requires trained personnel, has poor sensitivity in low-intensity infections, and detects only active infections, making it unsuitable for FGS diagnosis or surveillance. To address these diagnostic limitations, the WHO released a Target Product Profile (TPP) to guide development of new point-of-care (POC) diagnostics to support transmission interruption and surveillance (TI&S) after mass drug administration. In response, DDTD is driving a joint initiative to develop serological POC test(s) for *Sh* detection, which represent an appropriate and feasible solution to aid programmatic decision making in TI&S settings, as well as allowing detection of prior *Sh* infection to support FGS diagnosis. Eleven candidate protein biomarkers were identified through systematic literature review and partner collaboration before evaluation at two independent sites using multiplex bead assays (MBA). Each protein was expressed in CHO and *E.coli* systems and tested against two geographically distinct endemic settings including high-, medium-, and low-intensity *Sh*-positive samples, as well as *S. mansoni*-positive and uninfected samples. MBA performance informed down-selection of the six most promising antigens for lateral flow assay (LFA) development. Prototype LFAs were designed to detect IgG1 or IgG4 antibody responses to the six antigens with initial screening identifying optimal antigen and detector antibody pairs for specific use cases. Further evaluation with clinical samples showed 91% sensitivity (n=55) and 100% specificity (n=100) for IgG4 detection, while IgG1 detection yielded 87.3% sensitivity and 98% specificity across the same sample set. Consequently, DDTD has prioritized four antigens for optimization, with a goal of meeting the single-line test TPP target of 88% sensitivity and 99.5% specificity in a given population.

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DIAGNOSTIC EFFICACY OF DETECTING INFECTION AMONG SCHOOL CHILDREN AFTER MASS DRUG ADMINISTRATION (MDA) IN ZAMBIA COMPARISON OF SIX DIFFERENT DIAGNOSTICS

Brittany Pulkkila[1], Miriam Price[1], Sarah Alhakimi[1], Chummy Sikasunge[2], James C.L. Mwansa[3], **Nilanjan Lodh**[1]

[1]Department of Medical Laboratory Science, Marquette University, Milwaukee, WI, United States, [2]Department of Para-clinical Studies, University of Zambia, Lusaka, Zambia, [3]Lusaka Apex Medical University, Lusaka, Zambia

Schistosomiasis in Africa is a major Neglected Tropical Disease (NTD) with an estimated 250 million people infected. Diagnosing this NTD is difficult because in Africa, two of the main human species of schistosomes, *Schistosoma mansoni* and *S. haematobium*, are often sympatric. Detecting both species infection by current parasitological and immunological methods has low sensitivity, providing false positive results, especially after mass drug administration (MDA) via praziquantel. To address these shortcomings, we have detected both species from a single urine sample by six different diagnostic tests (including WHO-recommended ones) among school children in Zambia after MDA. This cross-sectional study included 130 school children (8-16 years) in Chongwe and Siavonga Districts in Zambia. Kato-Katz (KK) and circulating cathodic antigen (CCA) for *S. mansoni*, and urine filtration and hematuria (for *S. haematobium*) were conducted in Zambia. Species-specific cell-free repeat DNA fragments were amplified in the USA for both species from filtered urine samples by polymerase chain reaction (PCR) and loop-mediated isothermal amplification (LAMP). Our approach detected five times more positive cases by PCR (94) and LAMP (110) than by KK (0) and CCA (11) for *S. mansoni*. For S. *haematobium*, the outcome was similar against hematuria (PCR:78, LAMP: 80, and hematuria: 3) and even better against urine filtration (0). There were no false positives produced by LAMP. For *S. mansoni*, LAMP was the best indicator of disease prevalence (87.69%) with better sensitivity (96.49%) and specificity (100%). This was followed by PCR, CCA, and KK. Similarly, for *S. haematobium*, LAMP was the best method with 89.89% sensitivity, 100% specificity, and 68.46% disease prevalence, followed by PCR, hematuria, and urine filtration. In conclusion, The outcomes emphasize the need for a more accurate way of testing and identifying symptomatic and asymptomatic people infected with schistosomiasis, especially during MDA control interventions.

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PERFORMANCE, TIME, AND COST OF A POOLED TESTING STRATEGY FOR DETECTING AND QUANTIFYING *SCHISTOSOMA HAEMATOBIUM* INFECTION

Abraham Degarege[1], Berhanu Erko[2], Yohannes Negash[2], David M. Brett-Major[3], Bruno Levecke[4], Abebe Animut[5], Broadhurst M. Jana[1], Tzeyu Michaud[1], Christopher R. Bilder[6]

[1]University of Nebraska Medical Center, Omaha, NE, United States, [2]Aklilu Lemma Institute of Health Research, Addis Ababa University, Ethiopia, Addis Ababa, Ethiopia, [3]College of Public Health, University of Nebraska Medical Center, Omaha, NE, United States, [4]Ghent University, Merelbeke, Belgium, [5]Addis Ababa University, Addis Ababa, Ethiopia, [6]University of Nebraska-Lincoln, Linkoln, NE, United States

This study examined the performance time and cost of pooled testing for detecting and quantifying *Schistosoma haematobium* infection in urine samples in field settings. Between November 2024 and May 2025, urine samples were collected from school-aged children in three endemic regions of Ethiopia and examined for S. haematobium eggs using individual testing (IT), hierarchical pooled testing (HPT), and array pooled testing (APT). Diagnostic performance was assessed by comparing pooled testing results with combined IT and HPT or IT and APT reference standards. Individual testing showed the highest sensitivity for detecting S. haematobium (92.3% when IT and HPT used as reference or 94.4% when IT and APT used as reference). Sensitivity was moderately high for HPT (79.2%) and markedly reduced for APT (44.7%), particularly at lower infection intensities. Detection

probability in pooled samples increased with higher mean egg counts in individual specimens ($p < 0.001$). Egg count estimates from pooled testing correlated with individual testing, with stronger agreement for HPT (ρ=0.84) than APT (ρ=0.63). Compared with IT, HPT reduced sample processing and examination time by ~29.1% and cost by ~67.5%, while APT reduced time and cost by ~60.3% and ~81.8%, respectively. In conclusion, hierarchical and array pooled urine testing substantially improve efficiency of *S. haematobium* surveillance by reducing laboratory time and costs, with trade-offs in sensitivity compared to individual testing.

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DIAGNOSTIC PERFORMANCE OF A PROTOTYPE VISUAL FLOW-S ASSAY IN A KENYAN FIELD SETTING: A LARGE-VOLUME URINE CIRCULATING ANODIC ANTIGEN (CAA) POINT-OF-CARE TEST FOR SCHISTOSOMIASIS

Dylan Duerre[1], Cody Carrell[1], Mitch Geiger[1], Pytsje T. Hoekstra[2], Govert van Dam[2], Maurice R. Odiere[3], Fredrick Rawago[3], Gladys Odhiambo[3], Brianna Mullins[1], Jay Warrick[1]

[1]Salus Discovery, LLC, Madison, WI, United States, [2]Leiden University Medical Center, Leiden, Netherlands, [3]Centre for Global Health Research, Kenya Medical Research Institute, Kisumu, Kenya

Accurate, sensitive, and field-deployable diagnostics are essential to support control and elimination of schistosomiasis. Current diagnostics, including microscopy, lack sensitivity, particularly in detecting low intensity infections. Detection of circulating anodic antigen (CAA), a worm-derived biomarker present in serum and urine of individuals infected with *Schistosoma* parasites, can offer an alternative, but to obtain ultra-sensitivity, pre-concentration has restricted testing to a laboratory-based platform. A novel, low-cost, disposable and field-deployable device (Flow-S) has been developed which allows integration of passive, large-volume pre-concentration of CAA in urine and subsequent lateral flow (LF) detection. The workflow requires less than one minute of hands-on time, no pipetting or centrifugation, and can be performed by minimally trained field-workers. The first prototype used fluorescent UCP technology to detect CAA on an LF assay, requiring a portable, battery-powered reader. In a ~150-person field study in Tanzania, Flow-S achieved >93% sensitivity and >96% specificity, meeting the WHO TPP requirements for monitoring and evaluation of schistosomiasis (≥60% sensitivity, ≥95% specificity). However, dependence on a reader remains a major limitation. Therefore, Flow-S has been adapted to enable a visual readout, replacing the UCP with a visual label. The prototype visual Flow-S device will be prospectively evaluated in Kenya across *Schistosoma mansoni* (N=200) and *Schistosoma haematobium* (N=200) endemic settings. The performance of the Flow-S visual read-out will be assessed against a panel of diagnostics using a composite reference standard and latent class analysis. Field testing and data analysis will be finalized by July, 2026 and final results will be shared at ASTMH in November, 2026.

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THE UTILITY OF SS-NIE-1 IN ANTIGEN DETECTION AND ITS POTENTIAL AS A MOLECULAR MARKER FOR *STRONGYLOIDES STERCORALIS* DIAGNOSIS

Robertine Lontuo Fogang[1], Sasisekhar Bennuru[2], Thomas Nutman[2]

[1]National Institutes of Health, Rockville, MD, United States, [2]National Institutes of Health, Bethesda, MD, United States

Strongyloidiasis remains underdiagnosed due to the limited sensitivity and practicality of stool microscopy, and other available diagnostic tools. *Strongyloides stercoralis* (Ss)-NIE-1, a secreted, 31-kDa immunodiagnostic antigen derived from Ss L3 larvae has been widely used in serological assays for Ss-specific antibody detection. Its utility, however, for direct antigen detection has not been explored fully nor has its potential as a target for molecular diagnosis. The present study evaluates the potential of Ss-NIE-1 as a target for immunological and molecular diagnosis of Ss. Multiple monoclonal antibodies (MAbs) against recombinant Ss-NIE-1 were generated as were mono-specific high-titer polyclonal antibodies. These antibodies were used to develop multiple immunoassays for Ss-NIE-1 antigen detection. The most optimal (using 2 Mabs against different epitopes with Ss-NIE-1) showed a limit of detection (LOD) of 6ng/mL and was able to detect antigen in stool samples and in plasma/serum from ~50% of samples from Ss-infected patients. In parallel, the DNA sequence encoding Ss-NIE-1 was used to design primers, probes and gRNAs for two isothermal recombinase polymerase amplification (RPA) assays (RPA EXO and RPA- CRISPR/Cas12a) with visual readouts. The LOD for these 2 assays were 1fg/µL for the EXO RPA and 500fg/µL for RPA-CRISPR/Cas12a. Neither assay showed cross-amplification with other closely related helminths. When applied to clinical stool samples, the RPA assay detected Ss DNA in 4/6 qPCR-positive samples, whereas the RPA-CRISPR-Cas12a assay detected 3/6. While larger validation studies are needed for determination of clinical thresholds, these findings indicate that Ss-NIE-1 has diagnostic utility beyond antibody-based serology and may serve as the basis for both antigen-based and molecular detection of Ss at the point of patient contact

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PERFORMANCE OF CLIA-VALIDATED TRIPLEX ELISA FOR DETECTION OF ANTIBODIES TO *TAENIA SOLIUM* CYSTS IN HUMAN SERUM

Nadezhda Karaseva[1], Amy Songvilay[1], Drew Miller[1], Yevgeniy Studentsov[1], Elise O'Connell[2], Andrew E. Levin[1]

[1]Kephera Diagnostics, Framingham, MA, United States, [2]National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States

Neurocysticercosis (NCC) caused by *Taenia solium* infection of the central nervous system is responsible for about one third of global cases of epileptic seizures, and has been reported increasingly in the U.S. and Europe as a byproduct of immigration and travel. Diagnosis remains challenging, particularly in settings where imaging is limited or inconclusive. Serologic assays can be useful in corroborating or supplementing imaging information to distinguish epilepsy caused by NCC from other causes. The triplex NCC ELISA is a laboratory assay, which utilizes recombinant versions of three antigens (GP50, T24H, and Ts18var3) that represent the major antigen groups in the CDC gold standard Enzyme Immunotransfer Blot (EITB) assay as reported previously. We have validated an in-house version of the triplex ELISA for use in Kephera's CLIA/CAP accredited laboratory as a laboratory-developed test. We then evaluated assay performance on serum samples from patients originating in the U.S. and Peru with a diagnosis of NCC based on imaging, clinical history and EITB results. The assay demonstrated an overall sensitivity of 97.5% (116/119) and specificity of 97.5% (195/200) in a retrospective study with serum samples from NCC patients and healthy blood donors. Within the NCC patient group, the sensitivity for patients with subarachnoid cysts was 98.2% (56/57), for single cysts, 93.3% (14/15), and for multiple parenchymal cysts, 97.9% (46/47). Overall, the NCC ELISA demonstrated high diagnostic performance across clinically relevant NCC subtypes, supporting its use as a diagnostic aid in evaluation of patients with symptoms consistent with NCC. Further development and optimization of an algorithm for reflex testing of sera against the separate ELISA antigens in order to identify patients with viable cysts is underway.

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IMPLEMENTING COMMUNITY-BASED SNAIL CONTROL FOR SCHISTOSOMIASIS TRANSMISSION INTERRUPTION IN RWANDA: EVIDENCE FROM BUGESERA AND RUHANGO

Eric Niyongira[1], Nooliet Kabanyana[1], Ladislas Nshimiyimana[2], Jeanne Uwizeyimana[3], Louis Ngabonzima[1], Ivy Sempele[4], Alison Ower[4], Karen Palacio[4], Khadime Sylla[5], Eugene Ruberanziza[4]

[1]Rwanda NGOs Forum on HIV/AIDS and Health Promotion, KIGALI, Rwanda, [2]Rwanda Biomedical Centre, Kigali City, Rwanda, [3]Heart and Sole

Afica- Mwele Malecela Mentorship program (a mentee), Kigali, 1, Rwanda, [4]The END Fund, New York, NY, United States, [5]Université Cheikh Anta Diop de Dakar, Dakar, Senegal

In Rwanda, intestinal schistosomiasis (SCH) remains a public health concern despite sustained preventive chemotherapy through mass drug administration(MDA). Transmission persists in focal marshlands, indicating that MDA alone is insufficient for the Interruption of Transmission (IoT) in high-risk ecological settings. However, implementing community-based snail control remains limited. This study aimed to operationalize snail control within Rwanda's IoT strategy by integrating malacological evidence with environmental impact assessment (EIA). We conducted implementation research in Bugesera and Ruhango districts, combining a cross-sectional malacological survey, EIA, and intervention rollout. Four high-water contact marshland sites were purposively sampled as transmission hostpots. Teams used standardised scoop-net collections, identified species morphologically, and assessed Biomphalaria for cercarial shedding. EIA informed a cascade training model to operationalize sector-level snail control interventions, prioritizing environmental modification measures such as vegetation clearing, improvement of drainage and irrigation channels. A total of 2,157 snails were collected, of which 1,504 (69.7%) were Biomphalaria. Kanyegenyege contributed 1,096 snails and recorded the highest density, with 0.7% shedding schistosome cercariae. Snail control interventions were operationalised across 24 sectors through a cascade training approach involving 35 trainers of trainers and 70 peer educators, reaching 492 individuals from high-risk groups, including farmers and fishermen. Malacological and environmental assessments enabled the identification of transmission hotspots and supported the integration of community-based snail control within routine district planning systems. These findings provide proof of concept that environmentally informed, community-based snail control can be operationalized within decentralized health structures and contribute to strategies aimed at achieving SCH elimination as a public health problem and progressing towards IoT in focal high-risk settings in Rwanda.

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REFRAMING ASCARIASIS RISK: AUTOCHTHONOUS CASES IN HOUSTON, TEXAS AND STATEWIDE EPIDEMIOLOGIC TRENDS

Nina L. Tang[1], Megan M. Duffey[1], Ian P. Francis[1], Rania S. Milleron[2], Stephen L. White[2], Maria Elena Bottazzi[1], Jill Weatherhead[3]

[1]Baylor College of Medicine, Houston, TX, United States, [2]Texas Department of State Health Services, Austin, TX, United States, [3]National School of Tropical Medicine Baylor College of Medicine, Houston, TX, United States

Ascariasis is the most common human helminth infection worldwide, causing significant global morbidity. Although historically associated with low- and middle-income countries, ascariasis is increasingly recognized in geographically diverse resource-limited settings. In the United States, parasitic infections disproportionately affect vulnerable populations in subtropical environments, including the Gulf Coast where pediatric autochthonous ascariasis has been previously reported. We present two additional cases of autochthonous ascariasis in Houston, Texas, and summarize state-level epidemiologic data from 2016-2022. Case 1 was a 20-month-old female who presented after passing a roundworm identified as *Ascaris*. She was otherwise asymptomatic and diagnosed with uncomplicated ascariasis that was treated with a single dose of albendazole. Case 2 was an 18-month-old female with 4 days of frequent, large, watery stools that contained roundworms on the second day of illness. She was treated with a single dose of albendazole. However, she continued experiencing significant emesis (20+ episodes) and diarrhea (5+ episodes) over the subsequent days, each containing large quantities of roundworms identified as *Ascaris*. On repeat presentation, she required rehydration therapy but otherwise had an unremarkable physical exam. Both patients were born in Houston with no local or international travel history, sick contacts, or known exposures to pigs or pig manure. However, both patients resided in mobile homes with septic tanks in communities with high social vulnerability, underscoring the known association between parasitic infections and disadvantaged communities. Between 2016 and 2022, the Texas Department of State Health Services received 184 confirmed cases of ascariasis, of which 15 (8.1%) were autochthonous. These findings highlight local transmission within Houston with implications for similar communities within the United States, especially along the Gulf Coast. Together, these findings support reframing ascariasis risk in terms of environmental and socioeconomic conditions rather than national income.

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DIAGNOSTIC ACCURACY AND PROGRAMME MONITORING VALIDITY OF POC-CCA (POINT OF CARE CIRCULATING CATHODIC ANTIGEN) VERSUS KATO-KATZ FOR *SCHISTOSOMA MANSONI* BEFORE AND AFTER PRAZIQUANTEL: A NESTED COMPARISON WITHIN A RANDOMISED TRIAL IN PRU-EAST DISTRICT, GHANA.

Samuel Adusi Poku[1], Dennis Adu-Gyasi[2], Mathilda Tivura[2], Anthony Ibrahim Siibu[2], Elvis Wilson[2], Richmond Kessie[2], Elisha Adekunle Adeniji[2], Eliezer Ofori Odei-Lartey[2], Stephaney Gyaase[2], Tetteh Richard[2], Lucy Paintain[3], Tawiah Theresa[2], Paul Snell[3], Brian Greenwood[3], Daniel Chandramohan[3], Alexander Manu[1], Muhammed Olanrewaju Afolabi[3], Kwaku Poku Asante[2]

[1]Department of Epidemiology and Disease Control, University of Ghana, School of Public Health, Accra, Ghana, [2]Kintampo Health Research Centre and Development Division, Kintampo North, B/A, Ghana, [3]London School of Hygiene & Tropical Medicine, London, United Kingdom

Ghana targets schistosomiasis elimination by 2030, relying on Kato-Katz (KK) microscopy to monitor Praziquantel MDA effectiveness. We compared the diagnostic performance of POC-CCA and KK before and after praziquantel in a schistosomiasis-endemic setting in central Ghana. We enrolled 1,421 school-aged children in a cluster-randomised controlled trial in Pru-East District (Clinicaltrials.gov NCT06182176). Pre- and post-intervention stool and urine samples were tested at 5-month intervals using duplicate KK slides and POC-CCA dipstick. POC-CCA validity parameters were assessed against KK as the reference standard. Differential praziquantel effects were evaluated by difference-in-differences (DiD) mixed-effects logistic regression, adjusted for age, sex, ethnicity, and wealth index. Relative to KK, POC-CCA sensitivity was 66.7%, specificity (30.5%), positive predictive value (0.2%), and negative predictive value (99.8%). Near-zero correlation was observed (Spearman r = –0.003; 95% CI: –0.06 to 0.05). Kappa analysis yielded expected agreement (30.62%), observed agreement (30.61%), and kappa = –0.0002 (95% CI: –0.003 to 0.003). At baseline, KK detected 3 cases (0.2%) versus 987 by POC-CCA (69%); KK missed the *S. mansoni* cases in the intervention arm while POC-CCA detected 487 (69%). At endline, KK detected no cases. POC-CCA showed a 30% (69%→39%) reduction in positivity after praziquantel administration versus 22% (69%→47%) in controls. DiD regression confirmed a significant differential treatment effect (adjusted OR: 0.72; 95% CI: 0.51-0.96; p=0.042), representing a 28% reduction in odds of *S. mansoni* positivity attributable to praziquantel. Integrating POC-CCA with KK would strengthen programme monitoring and advance schistosomiasis elimination by 2030. The near-zero correlation and kappa values underscore the need for a more sensitive reference standard to accurately evaluate POC-CCA performance.

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RESULTS OF STOP-TREATMENT ASSESSMENTS FOR ONCHOCERCIASIS IN UPPER MADI MID NORTH FOCUS, UGANDA

Edson Byamukama[1], Lauri Bernard[2], David Oguttu[3], Edridah Tukahebwa Muheki[1], Elisa Byamukama[1], Christine Nahabwe[3], Monica Kembabazi[3], Alfred Mubangizi[3], Thomas R. Unnasch[4], Gregory S. Noland[2]

[1]The Carter Center - Uganda, Kampala, Uganda, [2]The Carter Center, Atlanta, GA, United States, [3]Ministry of Health, Kampala, Uganda, [4]University of South Florida, Tampa, FL, United States

The Madi Mid North (MMN) onchocerciasis focus in northern Uganda comprises 12 districts and borders the Republic of South Sudan. Permanent residents of MMN have received mass drug administration (MDA) with ivermectin for over 30 years. In 2023, the Uganda Onchocerciasis Elimination Expert Advisory Committee (UOEEAC) reclassified 7 districts of lower MMN sub-focus as "transmission interrupted" after epidemiologic and entomologic surveys met WHO stop-MDA criteria. However, the committee advised that the 5 districts of upper MMN sub-focus (Moyo, Adjumani, Amuru, Kitgum and Lamwo) continue MDA due to the risk of cross border transmission and the presence of refugee settlements with inhabitants from onchocerciasis-endemic areas in South Sudan and Sudan. In 2025, an epidemiological assessment of children ages 5-9 years was conducted in the upper MMN sub-focus, including children from Adjumani refugee settlements. Parishes were randomly selected using a multi-stage stratified sampling scheme and children per parish were sampled proportional to parish size. Blood samples were collected and tested for Ov16 antibodies using ELISA. Out of 6,344 samples (including 2,097 from refugee settlements), 7 children (0.11%, crude 95% CI 0.03-0.19%) from Adjumani refugee settlements were seropositive. All 7 tested negative for *Onchocerca volvulus* microfilariae by confirmatory skin snip PCR. Five children were born in the settlement (4 had never visited South Sudan), and 2 arrived at ≤ 2 months of age. Entomological monitoring was also conducted in all districts of upper MMN sub-focus, though not covering all areas of Adjumani refugee settlements. All *Simulium* flies collected in 2022-2023 (133 pools from 1,760 flies) and 2024-2025 (128 pools from 9,273 flies) tested negative for O-150 parasite DNA by conventional PCR (95% UCL, 0.2/2000 flies). The presence of Ov16-positive children with no reported travel history to other endemic areas suggests local onchocerciasis transmission may persist - especially near refugee settlement areas. Consequently, MDA was launched in the Adjumani refugee settlements in 2025 along with enhanced vector monitoring.

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NO EVIDENCE OF ONCHOCERCIASIS RECRUDESCENCE MORE THAN TWO YEARS AFTER STOPPING MASS DRUG ADMINISTRATION WITH IVERMECTIN IN NORTHERN GHANA

Andrew Abbott[1], Joseph Opare[2], Elizabeth Mary Wendt[1], Odame Asiedu[2], Ellen J. Doku[2], Ben Masiira[3], Thomson Lakwo[3], Ernest Kenu[4], Gifty Boateng[5], Florence Agyemang-Bioh[5], Rexford Adade[5], Karana Wickens[1], E. Scott Elder[1], Stephen Lindstrom[1], Paul T. Cantey[1]

[1]U.S. Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Neglected Tropical Diseases Program, Ghana Health Service, Accra, Ghana, [3]African Field Epidemiology Network, Kampala, Uganda, [4]African Field Epidemiology Network, Accra, Ghana, [5]National Public Health and Reference Laboratory, Ghana Health Service, Accra, Ghana

Mass drug administration (MDA) with ivermectin is the main strategy for eliminating river blindness. The current World Health Organization Ov16 serological threshold for stopping MDA is <0.1% at the upper 95% confidence interval (CI) in children aged <10 years, which may be too stringent. A study in 4 districts of Northern Ghana, under MDA for > 15 years (last in 2021), is evaluating a higher serological threshold of <2%. Baseline serologic and entomologic studies conducted in 2022 found Ov16 seroprevalence among 1,899 children aged 5-9 years of 1.3% (95% CI 0.7-2.3%) by Ov16 IgG4 rapid diagnostic test (RDT) and 0.09% (95% CI 0.02-0.55%) by Ov16m enzyme-linked immunosorbent assay (ELISA); prevalence of infective black flies by quantitative polymerase chain reaction (qPCR) was 0.013% (95% CI 0.003-0.380%). Serological and entomological monitoring for recrudescence were conducted in 2024 and 2025. Ten previously unsampled communities were selected each monitoring year based on proximity to known black fly breeding sites or to communities with the most RDT-positive children. In each community, 50-100 randomly selected children aged 5-9 years provided dried blood spots for analysis by RDT and Ov16m ELISA. If <50 children were present, the next nearest community was also sampled. In 2024, black fly catching occurred in the same 10 sites as baseline. In 2025, 6 of these sites were included. DNA was extracted from pooled fly heads and analyzed by O150 qPCR with confirmatory ND5 qPCR. In 2024, 4 (0.5%) of 783 children enrolled from 3 communities had positive results (0-2.40% seroprevalence by community); 1 child was positive by RDT and ELISA and 3 were positive by ELISA alone. In 2024, 12,141 black flies were collected; none were confirmed positive by qPCR. In 2025, 811 children aged 5-9 years were sampled and 1,835 black flies were captured (results pending). The next step is to conduct a full serological and entomological survey similar to baseline. Serological and entomological results from specimens collected > 2 years after the last MDA show no evidence of recrudescence, suggesting transmission may have been interrupted.

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A LONGITUDINAL EVALUATION OF HIGHER SEROLOGIC THRESHOLD FOR STOPPING MASS DRUG ADMINISTRATION IN ONCHOCERCIASIS ELIMINATION IN THE TUKUYU FOCUS, TANZANIA, 2020-2025

Andreas M. Nshala[1], Rebecca Chancey[1], Scott E. Elder[1], Stephen Lindstrom[1], Paul M. Hayuma[2], Ben Masiira[3], Lakwo T. Luroni[4], Erick J. Mgina[5], Clara Jones[6], Hong Zhou[1], Akili K. Kalinga[7], Paul T. Cantey[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]National Institute for Medical Research - NIMR, Tanga, United Republic of Tanzania, [3]African Field Epidemiology Network (AFENET), Kampala, Uganda, [4]Ministry of Health Uganda, Kampala, Uganda, [5]National Institute for Medical Research - NIMR, Dar es salaam, United Republic of Tanzania, [6]Dodoma, United Republic of Tanzania, [7]NIMR, Dar Es Salaam, United Republic of Tanzania

Onchocerciasis elimination strategies rely primarily on repeated annual mass drug administration (MDA) with ivermectin over multiple years to interrupt transmission. The World Health Organization (WHO) recommends that programs consider stopping MDA only when both entomological and seroprevalence criteria are met. However, the recommended serologic threshold of <0.1% is operationally challenging and may be more stringent than necessary. We evaluate whether a serologic threshold of ≤2% could be used to safely stop MDA in Tukuyu focus, Tanzania. A 5-year longitudinal study included baseline (2020), 2 assessments during 3 years of post-treatment surveillance (PTS) (2022, 2024), and endline (2025). At baseline, 2,084 children aged 5-9 years from 30 villages were tested for Ov16 antibodies. During PTS, 809 and 806 children from 8 purposively selected first-line villages were tested in 2022 and 2024, respectively. At endline, 2,074 children were tested. Dried blood spots were analyzed by Ov16 rapid diagnostic test (RDT), and Ov16 and Ov16m enzyme-linked immunosorbent assays (ELISA). Concurrently, *Simulium damnosum* blackflies were collected during a 5-month breeding season at 8 sites using human landing collections and tested by quantitative polymerase chain reaction (qPCR) for O-150 and OvND5. Baseline seroprevalence was 1.21% by Ov16 ELISA, 0.14% by Ov16m ELISA, and 0.05% by RDT. In 2022 and 2024, seroprevalence was 0.12% by both RDT and Ov16m ELISA. At endline, in 2025, all 2,074 children were RDT-negative. Entomologic assessments during baseline were inconclusive but found no evidence of *Onchocerca volvulus* infection in 3,726 blackflies. Results from the 11,947 blackflies in 2024, and 8,510 in 2025 are pending confirmatory testing. Based on

these serological and entomological findings, the Tukuyu focus stopped MDA according to the study criteria, completed the three-year WHO-recommended PTS period, and underwent a final evaluation with no evidence of recrudescence. These results provide additional evidence that adjusting the serologic threshold for stopping MDA to ≤2% may be safe, without resulting in residual transmission.

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LYMPHATIC FILARIASIS TRANSMISSION AT SPOT-CHECK SITES IN SIX ENDEMIC DISTRICTS OF NEPAL AFTER TWO ROUNDS OF MASS DRUG ADMINISTRATION USING IVERMECTIN, DIETHYLCARBAMAZINE, AND ALBENDAZOLE

Ram Kumar Mahato[1], Gokarna Dahal[2], Shashi Kandel[1], Satyaraj Paudel[1], Anish Chaudhary[3], Prakash Shakya[4], Bhim Prakash Devkota[5], Rabindra Khaniya[4], Bhim Prasad Sapkota[6], Krishna Prasad Paudel[7], Bijay Bajracharya[8], Deena Shrestha[9], Chandra Bhal Jha[1], Roshan Neupane[1], Kenza Bennani[4]

[1]Epidemiology and Disease Control Division, Kathmandu, Nepal, [2]Epidemiology and Disease Control Division, Ministry of Health, Kathmandu, Nepal, [3]National Public Health Laboratory, Kathmandu, Nepal, [4]World Health Organization, Kathmandu, Nepal, [5]Department of Health Services, Kathmandu, Nepal, [6]Ministry of Health and Population, Kathmandu, Nepal, [7]Ministry of Health and population, Kathmandu, Nepal, [8]CHDS, Kathmandu, Nepal, [9]Center for Health and Disease Studies Nepal, Kathmandu, Nepal

Nepal aims to eliminate lymphatic filariasis (LF) by 2030. Mass drug administration (MDA) has been completed in 56 of 64 endemic districts. In 2024, six districts with persistent LF (≥2% antigen prevalence) conducted two rounds of MDA using the triple-drug regimen (Ivermectin, Diethylcarbamazine, Albendazole—IDA), achieving >65% coverage. An Epidemiological Monitoring Survey (EMS) was conducted to assess the impact on LF transmission. A cross-sectional EMS was conducted nine months post-MDA in 12 evaluation units (EUs) across six districts, with two sites per EU (n=24). A total of 7,343 individuals aged ≥20 years were selected through multi-stage sampling, ensuring ≥300 samples per site. Demographic and MDA participation data were collected. All participants underwent antigen testing; antigen-positive cases were further tested for microfilariae (MF) using night blood samples. Statistical analysis included non-parametric tests, Chi-square/Fisher's Exact tests, and multivariate logistic regression. Nine of 12 EUs achieved <1% MF prevalence, meeting WHO criteria, while three EUs failed (≥1% MF in at least one site). Antigen and MF prevalence were strongly associated with participation in the latest MDA (AOR=0.477; 95% CI: 0.385-0.591 and AOR=0.089; 95% CI: 0.017-0.464). MDA uptake was influenced by age (<40 years, AOR=0.72), sex (female, AOR=1.438), cross-border residence (AOR=0.616), and occupation (AOR=1.144). MF prevalence was independently associated with younger age (AOR=0.225) and female sex (AOR=0.20). Overall antigen and MF prevalence were 4.47% and 0.34% (ratio 13:1). In conclusion, significant progress toward LF elimination is evident, with most EUs meeting WHO thresholds after two IDA rounds. However, ongoing transmission in some areas is linked to lower MDA participation, especially among males, younger adults, and cross-border populations. Targeted strategies to improve coverage and compliance, particularly in border areas, are essential.

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POST-TREATMENT SURVEILLANCE FOR LYMPHATIC FILARIASIS WITH INTEGRATED MALARIA DIAGNOSTICS IN OUEST DEPARTMENT, HAITI, 2025

Hannah Lawinger[1], Luccene Desir[1], Mérilien Jean-Baptiste[2], Dérival Fritz-Gérald[2], Stephane Docteur[1], Nishant Kishore[1], Gregory S. Noland[1], Marc-Aurèle Telfort[2], Karen E.S. Hamre[1]

[1]The Carter Center, Atlanta, GA, United States, [2]Ministère de la Santé Publique et de la Population, Port-au-Prince, Haiti

Haiti is 1 of 3 countries in the Americas endemic for lymphatic filariasis (LF), a parasitic disease caused by *Wuchereria bancrofti* transmitted by *Culex* mosquitoes; it is also endemic for *Plasmodium falciparum* malaria transmitted by *Anopheles*. In Ouest department, the districts of Petit-Goâve and Grand-Goâve halted LF mass drug administration (MDA) after each passed transmission assessment survey (TAS)-1 in 2017, along with Anse-à-Galets and Pointe-à-Raquette (on La Gonâve island) in 2019. In post-treatment surveillance, the World Health Organization (WHO) recommends repeated TAS among children ages 6-7 years to demonstrate infection remains below the target threshold (updated in 2025 from < 2% to < 1% antigen prevalence for *W. bancrofti*), at which transmission is considered unsustainable. In August 2025, the Haitian Ministry of Public Health and Population (MSPP) conducted community-based TAS-3 in 3 evaluation units (EUs) among these 4 districts. In the combined EU of Petit-Goâve and Grand Goâve, 0 (of 1,627) children tested positive for circulating filarial antigen (CFA) by Filariasis Test Strips (FTS), and in Pointe-à-Raquette, 3 (0.34%) of 872 children tested positive. Both results were at or beneath the critical cut-off values of 6 and 3, respectively, to pass TAS. In Anse-à-Galets, 5 (0.36%) of 1,380 children tested positive, surpassing the critical cut-off of 4, and failing TAS. The EU would have passed under former WHO criteria (critical cut-off = 16). All 5 children were born there suggesting ongoing transmission. Night blood samples were collected from CFA-positive children; none had circulating microfilariae detected by microscopy. Rapid testing for malaria (*Pf*/Pv) was integrated into the TAS: 5 (0.13%) of 3,722 children among the 3 EUs tested positive for *Pf* (2 in Petit-Goâve; 3 in Grand-Goâve). Each was referred to a health facility for treatment. MSPP successfully implemented an integrated community-TAS amidst widespread insecurity and continued progress toward its elimination goals with 66 (47%) of 140 communes now having passed TAS-3. MSPP intends to conduct 2 additional rounds of MDA in La Gonâve island.

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MAPPING GUINEA WORM DISEASE RISK TO INFORM ERADICATION STRATEGIES IN SUB-SAHARAN AFRICA

Edson C. Utazi[1], Somnath Chaudhuri[1], Shengjie Lai[1], Sujit K. Sahu[1], Kathryn Schaber[2], Obiora Eneanya[2], Adam Joseph Weiss[2]

[1]University of Southampton, Southampton, United Kingdom, [2]Guinea Worm Eradication Program, The Carter Center, Atlanta, GA, United States

High-resolution risk maps of infectious diseases such as Guinea worm disease (GWD, dracunculiasis) are crucial to guide the planning, implementation and monitoring of interventions, including water treatment with Abate™ and targeted surveillance. Despite their demonstrated utility, such maps remain unavailable for most GWD endemic countries, except Chad. Our study addresses this gap by developing a hybrid geostatistical-machine learning framework to produce 1x1 km risk maps of GWD, quantify associated uncertainties, and identify key drivers of environmental suitability across six endemic countries. We analysed host-level infection data from 2007 - 2025, comprising 22, 743 cases and infections, combined with a suite of geospatial environmental, climatic, socioeconomic and demographic covariates. We fitted spatiotemporal models for Chad, Cameroon and South Sudan, whereas spatial models were used for Angola, Ethiopia and Mali, due to lower case counts. All models were implemented in a Bayesian framework using the INLA-SPDE approach. Predictive performance was assessed using random, spatial, temporal and environmental cross-validation. Our results indicate persistent, broader spatial distribution of risk in Chad and Cameroon in the period from 2015 to 2021, particularly around the Chari and Logone rivers and the Salamat province, followed by a narrowing of high-risk areas in recent years. South Sudan shows marked declines in high-risk areas suggesting recent progress. Ethiopia, Angola and Mali exhibit pronounced spatial clustering of disease risk. In Chad and Cameroon, the estimated numbers of persons living in hotspot areas ranged between 3.83 million and 4.53 million during 2021 to 2025. Across the countries, key correlates of environmental suitability for GWD include temperature, vapour pressure, precipitation, proximity to waterbodies, conflict and access to healthcare. By identifying where transmission is most likely to persist or re-emerge, our analyses provide a practical decision-support tool to intensify interventions, close residual transmission foci, and accelerate progress towards GWD eradication.

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SIMULATION MODELING TO INFORM TARGET PRODUCT PROFILES FOR INTERVENTION DEVELOPMENT: THE CASE OF GUINEA WORM DISEASE

Hannah K. Smalley[1], Pinar Keskinocak[1], Maryann G. Delea[2], Obiora A. Eneanya[2], Jessica M. van Loben Sels[2], Alexandra Sack[2], Philip Ouakou Tchindebet[3], Adam Weiss[2]

[1]Georgia Institute of Technology, Atlanta, GA, United States, [2]The Carter Center, Atlanta, GA, United States, [3]Guinea Worm Eradication Program, Ministry of Public Health, N'Djamena, Chad

In the fight to eradicate Guinea worm disease (GWD), a 99.99% reduction in human cases has been achieved without the aid of a treatment, a vaccine, or diagnostics that can detect recent or prepatent infection. However, animal infections, specifically in domestic dogs, persist and have created challenges; additional tools and interventions may be needed to reach eradication. We use an agent-based simulation to inform intervention Target Product Profile (TPP) development for Guinea worm therapeutics by considering product characteristics in combination with potential deployment and response scenarios. In prior work, we investigated the potential benefits of a hypothetical diagnostic test for detecting pre-patent infections in dogs. Using data from the Chad Guinea Worm Eradication Program, we modelled dog infections considering the GWD life cycle, seasonality, environmental factors, and interventions. Here, we extended the model to evaluate the impacts of potential therapeutics. We evaluate three treatment types - adulticidal, larvicidal, and reducedfecundity - across multiple deployment strategies. We examined treatment efficacy levels and deployment conditions necessary for reducing GWD transmission. The findings indicate that the most effective therapeutic option depends on the deployment approach: the larvicidal is preferred when village-level treatment is initiated only after a worm emergence event is detected, whereas adulticidal and reducedfecundity treatments are more effective when paired with diagnosticbased Test & Treat strategies. Zero infections were achieved by five years with 80% efficacy for adulticidal or reduced fecundity treatment types using a Test & Treat strategy even with low tethering adherence. Zero infections were not achieved within 10 years when any treatment is deployed only after an emergence event, even at 100% efficacy without also having medium to high tethering adherence. These results highlight the importance of aligning therapeutic characteristics with operational deployment methods to support elimination of GWD in dogs in Chad and can guide TPP criteria and investment decisions.

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DEFINING VISCERAL LEISHMANIASIS TRANSMISSION HOTSPOTS IN KENYA TO GUIDE TARGETED ELIMINATION STRATEGIES

Paul Kibati[1], Daniel Mwiti Ringera[2], Mutono Nyamai[3], Robert Ofwete[4], Esther Muthoni Kinyeru[5], Cherinet Adera[6], Kebron Haile[7], Robert Karanja[8], Daniel Masiga[9], Damaris Matoke-Muhia[8], Duncan Ochol[10], Michael Muchangi[1], Michael Ofire Ofire[2], Wyckliff Peter Omondi[11], George Omondi Paul[12]

[1]Amref Health Africa, Nairobi, Kenya, [2]Nairobi, Kenya, [3]UNIVERSITY OF NAIROBI, Nairobi, Kenya, [4]9402, Kenya, [5]WHO Kenya, Nairobi, Kenya, [6]Drugs for Neglected Diseases Initiative, Nairobi, Kenya, [7]The END Fund, Wolcott, CT, United States, [8]Kenya Medical Research Institute, Nairobi, Kenya, [9]International Centre of Insect Physiology and Ecology, Nairobi, Kenya, [10]The END Fund, Nairobi, Kenya, [11]Ministry of Health, Nairobi, Kenya, [12]Centre for Epidemiological Modelling and Analysis, University of Nairobi, Nairobi, Kenya

Kenya is among the ten countries accounting for over 90% of global visceral leishmaniasis (VL) cases, with burden concentrated in arid and semi-arid regions. Transmission is driven by environmental and behavioral factors including expanding sandfly habitats, outdoor exposure, and population mobility, disproportionately affecting children who account for most cases. Although VL transmission is highly focal and clustered, interventions in Kenya have predominantly been implemented at broader administrative levels, constraining their effectiveness in disease control. To inform development of an evidence-based VL elimination strategy aligned with the WHO Eastern Africa framework (2023-2030), we conducted a spatial analysis of surveillance data from a retrospective review of the WHO integrated data platform and the Kenya Health Information System, with ward-level analysis to identify hotspots. Spatial heterogeneity was marked, with 20 wards representing less than 1.4% of approximately 1,450 wards accounting for about 60% of reported VL cases. These high-burden wards are concentrated in West Pokot, Wajir, Turkana and Baringo counties, with incidence exceeding 50 cases per 10,000 population in several locations. Entomological evidence indicates transmission driven primarily by *Phlebotomus martini*, associated with termite mound habitats and outdoor exposure, while detection of *Phlebotomus alexandri* in northern Kenya suggests evolving vector ecology and potential expansion of transmission risk. These findings demonstrate that VL burden in Kenya is highly concentrated within defined geographic foci. Targeting these wards provides an opportunity to intensify early case detection through active case finding, strengthen community sensitization, and expand access to diagnosis and treatment. Establishing entomological sentinel surveillance sites would generate context-specific data to guide vector control strategies. These findings support implementation of an integrated, geographically targeted intervention package to accelerate progress toward VL elimination as a public health problem by 2030.

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SPATIAL ANALYSIS OF LEPROSY REVEALS TRANSMISSION DECLINE ALONGSIDE PERSISTENT FOCAL HOTSPOTS IN NEPAL

Rabindra Baskota[1], Nanda Lal Bastola[2]

[1]Ministry of Health and Population, Nepal, Kathmandu, Nepal, [2]NLR, Kathmandu, Nepal

Leprosy transmission in Nepal has declined substantially over recent decades, yet persistent localized clusters continue to challenge the achievement of uniform elimination. We analyzed leprosy surveillance data from 137 municipalities in Koshi Province, between 2000 and 2025 using the Leprosy Elimination Monitoring Tool to characterize temporal and spatial transmission dynamics. Transmission status was assessed using proxy indicators including persistence of cases, occurrence of autochthonous child cases, and spatial clustering. We observed a marked decline in transmission, with transition from widespread endemicity in 2000 to predominantly non-endemic status by 2025. This decline was accompanied by increasing geographic clustering, with cases becoming concentrated in fewer municipalities. Despite overall progress, persistent transmission remained in the southern Terai belt. Spatial analysis revealed a contiguous high-risk corridor in these areas, alongside sparse and fragmented clusters in hill regions. Relative risk analysis identified one major high-risk cluster (RR=6.56), a localized micro-hotspot in Belaka (RR≈3.99), and several moderate clusters (RR≈1.6-2.0), indicating focal but ongoing transmission. We also observed apparent re-emergence patterns in some municipalities, characterized by fluctuations in case detection after periods of low transmission. However, these patterns were not consistent with known leprosy epidemiology and were more likely attributable to data inconsistencies, delayed detection, or population mobility rather than true biological resurgence. The increasing clustering and reduction in widespread transmission evidenced that ongoing interventions, including active case finding and chemoprophylaxis, are contributing to transmission decline. However, the persistence of hotspot areas indicates that uniform strategies are insufficient and require targeted interventions to interrupt transmission and achieve sustained elimination.

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EARLY SIGNALS OF FILOVIRUS OUTBREAKS

Merline R. Feero[1], David Heymann[1], Daniel G. Bausch[2]

[1]London School of Hygiene and Tropical Medicine, London, United Kingdom, [2]Centre for Infectious Disease Emergency Response (CIDER), National University of Singapore, Singapore, Singapore

Rapid recognition of filovirus disease (Ebola and Marburg virus infection) is essential for outbreak control, but early clinical and epidemiological cues that raise suspicion remain poorly characterized. We reviewed published and grey literature from five databases to characterize the first alerts of 46 community-based filovirus outbreaks from 1976-2025, including 99 reports. For outbreaks where report type was available, the majority (87%) were reported by physicians or hospital staff. Community alerts in conjunction with physicians/hospital staff accounted for 4%, while 2% were made by the community alone; no specific source was recorded for 7%. Alerts from HCWs arose from both single cases (63%) and case clusters (37%), while alerts from the community were usually after prolonged clusters of unusual disease and deaths. No alert arose from routine indicator-based surveillance. Single index cases were often (37%) family members of someone who had previously fallen ill, had occupational exposures to ill persons or zoonotic reservoirs (miners, hunters, factory workers), or were immediate family members of one of these occupations. The route of virus introduction was considered known in 61% of outbreaks. Presenting signs and symptoms of ill persons were generally non-specific. Hemorrhage was noted in 56% of cases, usually late in illness. Misdiagnosis as malaria or other common febrile illnesses was frequent. Although the sources of first alerts have not appreciably changed over time, the time from first alert to laboratory confirmation generally decreased, suggesting improved laboratory capacity. However, timelines from retrospectively identified first suspected case(s) to outbreak recognition were often prolonged. Clinical suspicion by frontline HCWs and event-based surveillance remain the dominant signals of filovirus outbreaks, but often occur too late for early detection and rapid control. We discuss strategies to accelerate recognition, including HCW training and knowledge sharing; enhanced indicator-based, community-led and One Health surveillance; and multiplex diagnostic approaches.

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SEROPREVALENCE OF NEGLECTED TROPICAL DISEASES, ARBOVIRUSES, VACCINE-PREVENTABLE DISEASES, AND ENTERIC PROTOZOA FROM A NATIONAL MULTI-PATHOGEN SEROSURVEY IN NIUE

Harriet LS Lawford[1], Grizelda Mokoia[2], Patricia Tatui[2], Misiona Nicolas[2], Andy Manu[2], Amanda K. Murphy[3], Tonia Marquardt[4], Leanne J. Robinson[5], Fiona Angrisano[5], Colleen L. Lau[1], Adam T. Craig[1]

[1]Centre for Clinical Research, Faculty of Health, Medicine, and Behavioural Sciences, The University of Queensland, Brisbane, Australia, [2]Department of Health, Alofi, Niue, [3]QIMR Berghofer Medical Research Institute, Herston, Australia, [4]Queensland Health, Brisbane, Australia, [5]Burnet Institute, Melbourne, Australia

Integrated serosurveillance (ISS) measures the seroprevalence of antibodies (Ab) against pathogen-specific antigens (Ag), providing insights into population immunity to vaccine-preventable diseases (VPDs) and exposure to infectious diseases (ID). We estimated the seroprevalence of 17 neglected tropical diseases (NTDs) (lymphatic filariasis [LF], yaws, trachoma, taeniasis, cysticercosis, strongyloidiasis), arboviruses (dengue, chikungunya, Zika), VPDs (measles, rubella, diphtheria, tetanus, pertussis, SARS-CoV-2), and enteric protozoa (giardiasis, cryptosporidium) in Niue, a Pacific island country, to inform ID control and elimination strategies. LF post-validation surveillance was embedded in a national WHO STEPwise survey in February-March 2025, demonstrating a novel integrated surveillance platform. Dried blood spots were collected from ≥3-year-olds and analysed using a multiplex bead assay (MBA) containing 26 Ag. Seroprevalence was estimated by age, sex, and recruitment site. Multivariable logistic regression assessed associations. 750/898 participants (83.5%) (median age 37 years; 53% female) had valid MBA results. NTD seroprevalence was low (0% [yaws] to 2.7% [cysticercosis]). Arbovirus exposure ranged from 3% (Zika) to 35% (dengue), increasing with age ($p<0.001$) and varying by recruitment site ($p<0.05$). Seroprevalence was ≥98% for measles, rubella and SARS-CoV-2. Diphtheria (89%) and tetanus (96%) seroprevalence were lower and declined with age ($p\leq0.02$), with age-sex interactions ($p\leq0.002$) indicating lower immunity among older females. Cryptosporidium seroprevalence was 69% and increased with age ($p<0.001$) while giardiasis remained low (4%). This study provides a national snapshot of multi-pathogen immunity and infection in Niue, demonstrating the value of MBA as a cost-effective tool for ISS. Findings indicate sustained control of targeted NTDs and high vaccination coverage, with evidence of waning immunity in specific subgroups. These findings support targeted arbovirus surveillance, further investigation of enteric transmission and age- and sex-specific vaccination strategies.

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EPIDEMIOLOGICAL AND CLINICAL CHARACTERISTICS OF MPOX CASES IN LIBERIA, 2017 - 2025

Emmanuel Dwalu[1], Bode I. Shobayo[1], Ralph W. Jetoh[1], Fahn M. Taweh[1], Julius S.M. Gilayeneh[1], Sia W. Camanor[1], Neima N. Candy[2], Peter A. Adewuyi[3], Lily M. Sanvee – Blebo[3], Faith K. Whesseh[3], Chukwuma D. Umeokonkwo[4]

[1]National Public Health Institute of Liberia, Monrovia, Liberia, [2]University of Liberia College of Health Sciences, Monrovia, Liberia, [3]African Field Epidemiology Network (AFENET), Liberia Office, Monrovia, Liberia, [4]African Field Epidemiology Network (AFENET), Kampala, Uganda

Mpox is an infectious viral disease with a recent rise in cases above threshold across Africa, prompting the Africa Centers for Disease Control and the World Health Organization to declare it a public health emergency. In Liberia, one confirmed case of Mpox constitutes an outbreak but there is limited information on the characteristics of the cases. We described the epidemiological and clinical characteristics of Mpox cases in Liberia and determined factors associated with mortality. We conducted an analytic cross-sectional study utilizing case-based Mpox surveillance data from the National Public Health Institute of Liberia, 2017 - 2025. All suspected cases were tested using Real-time Polymerase Chain Reaction at the National Public Health Reference Laboratory. We summarized the characteristics of the cases and examined factors associated with Mpox mortality at 95% confidence intervals and p-values < 0.05. Of the 2944 suspected cases reported, 87% (2571) were tested, and 56.6% (1455) confirmed Mpox positive. The median age was 30 years (IQR 21-38). Age 20-39 years (57.9%, 842), males (61.0%, 887), students (27.6%, 401), and urban Montserrado (67.6%, 984) recorded the highest confirmed cases. Rashes (69.6%, 1013), fever (46.3%,674), headache (29.0%, 422), and body aches (28.9%, 421) were the common symptoms. The median duration from symptom onset to admission was 4 (IQR:3-7) days, with 0.5% (8) case fatality. The outbreak began with sporadic cases in 2022 (0.4%, 6) but exponentially increased in 2025 (93.8%, 1365). Residing in Grand Gedeh (aOR = 20.5, 95% CI: 1.921-217.750) and >=7days duration from symptoms onset to admission (cOR = 6.6, 95% CI: 1.304 -33.623) were risk factors for mortality. The Mpox outbreak in Liberia affected young adult males and clustered in urban Montserrado. There was an increasing trend with the highest cases recorded in 2025. Rashes, fever, headache, and body aches were the common symptoms. We presented these findings at the Mpox Incident Management System meeting and recommended further studies to investigate reasons for the increase among young adult males, urban cluster in 2025, and risk factors for mortality.

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SOCIAL MIXING PATTERNS AND 171 EMPIRICAL CONTACT MATRICES FOR INFECTIOUS DISEASE MODELING IN SUB-SAHARAN AFRICA: A SYSTEMATIC REVIEW AND META-ANALYSIS

Francis B. Kengne[1], Faharudeen Alhassan[2], Jing Kersey[1], Kin On Kwok[3], Gerardo Chowell[2], Isaac Chun Hai Fung[1]

[1]Georgia Southern University, Statesboro, GA, United States, [2]Georgia State University, Atlanta, GA, United States, [3]The Chinese University of Hong Kong, Hong Kong, Hong Kong

Empirical social-contact matrices are essential for infectious disease modeling. However, evidence from SubSaharan Africa (SSA) remains fragmented. We conducted a systematic review and metaanalysis of SSA empirical social-contact surveys following PRISMA guidelines (PROSPERO CRD420261289085). Screening, data extraction, and quality assessment were performed independently by 2 reviewers. Individualparticipant data (IPD) were harmonized and analyzed using Bayesian negative binomial and logistic regression models to estimate the determinants of total, physical, and longduration contacts. Twentytwo studies conducted between 2010 and 2022 in 18 countries were included, yielding 171 empirical contact matrices published in our R ssamixr package. IPD from 16 surveys were analyzed. Children <15 years reported the highest daily contacts (Median =10, IQR = 7-14). Adults aged 15-44 years had modestly lower adjusted contact rates (adjCRR = 0.96, 95% CrI [0.94, 0.99]). Household size showed the strongest gradient: compared with 1-2person households, contact rates increased by 13% in 3-5person households (adjCRR = 1.13, 95% CrI [1.09, 1.17]), by 39% in 6-10person households (adjCRR = 1.39, 95% CrI [1.33, 1.44]), and by 51% in 11-plusperson households (adjCRR = 1.51, 95% CrI [1.42, 1.61]). Rural participants reported more contacts (Median = 11, IQR = 8-15) than urban participants (Median = 8, IQR = 5-12). Methodological variables showed weaker and more uncertain effects. Retrospective recall tended to underestimate contacts (prospective: Median = 10, IQR = 7-14 vs. retrospective: Median = 5, IQR = 3-8). Weekend contacts were almost the same as weekday contacts (adjCRR = 0.96, 95% CrI [0.94, 0.99]), but overall, they tended to be more physical and lasted longer across many subgroups. Empirical variations (age, household size, and rural-urban context) dominate SSA social mixing patterns. Most methodological differences exert limited influence except recall type and weekend effects. These findings provide an evidencebased foundation for selecting SSA contact matrices in infectious disease modeling.

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ZOONOTIC SPILLOVER OF PTEROPINE ORTHOREOVIRUS LINKED TO NIPAH-LIKE ILLNESS IN BANGLADESH

Ariful Islam[1], James Ng[2], Sharmin Sultana[3], Ellen Murphree[4], Md. Arif Khan[5], Pronesh Dutta[6], Wenxin Tian[7], Dubey Sunil Kumar Dubey[7], Reed F Johnson[8], Jade K. Forwood[1], Lisa E Hensely[4], Tahmina Shirin[9], Nischay Mishra[7]

[1]Gulbali Research Institute, Charles Sturt University, Wagga Wagga, NSW, Australia, [2]Center for Infection and Immunity, Mailman School of Public Health, Columbia University, New York, USA, NY, United States, [3]Institute of Epidemiology Disease Control and Research, Dhaka, 13, Bangladesh, [4]Zoonotic and Emerging Disease Research Unit (ZEDRU), National Bio and Agro-Defense Facility (NBAF), United States Department of Agriculture (USDA), Manhattan, KS, USA, Manhattan, KS, United States, [5]Institute of Epidemiology, Disease Control and Research (IEDCR), Mohakhali, Bangladesh, [6]Institute of Epidemiology, Disease Control and Research (IEDCR), Mohakhali, Dhaka-1212, Bangladesh, [7]Center for Infection and Immunity, Mailman School of Public Health, Columbia University, New York, NY, United States, [8]Zoonotic and Emerging Disease Research Unit (ZEDRU), National Bio and Agro-Defense Facility (NBAF), United States Department of Agriculture (USDA), Manhattan, KS, USA, Manhattan, NY, United States, [9]Institute of Epidemiology, Disease Control and Research (IEDCR), Mohakhali, Dhaka-1212, Australia

Pteropine orthoreoviruses (PRVs) are emerging bat-borne viruses previously associated with mild respiratory illness in humans. Between December 2022 and April 2023, 136 patients presenting with Nipah-like illness but tested negative for Nipah virus (NiV) were enrolled through Bangladesh (BD) national surveillance. Throat swabs were analyzed using capture-based high-throughput sequencing (VirCapSeq-VERT), followed by virus isolation, full-genome sequencing. We developed PRV-specific qPCR and partial S1 (P10 gene) consensus PCR assays for detection and genotyping. Concurrent wildlife surveillance sampled 326 fruit bats across four NiV-endemic districts and paired throat, and rectal swabs were screened using PRV-qPCR and PRV-P10-S1 PCR. Common respiratory viruses, including adenoviruses, herpesviruses, and rhinoviruses, were detected in NiV-negative patients. PRV was identified in 5 of 136 patients (3.7%), with three isolates successfully cultured, showing 93.7-100% average nucleotide identity (ANI) with known bat and human PRVs. All BD-Human-PRV positive patients lived within 25-250 km in central Bangladesh, without epidemiologic links, and consumed raw-date-palm-sap 3-14 days before illness onset. Three patients recovered fully, one developed persistent neurological sequelae, and one died after prolonged complications. PRV RNA was detected exclusively in *Rousettus leschenaultii* bats (17/101; 16.8%), with evidence of both oral and fecal shedding and geographic overlap with BD-Human-PRV cases. P10-S1 phylogenetic analysis showed bat-derived PRV from Bangladesh (BD-bat-PRV) with >99% ANI to BD-Human-PRV isolates. Whole-genome sequences of BD-bat-PRV showed 82-95% ANI with human isolates. These findings provide evidence of zoonotic spillover of PRV linked to consumption of date-palm sap contaminated with bat excreta and associated with severe Nipah-like illness. PRV should be included in the differential diagnosis of Nipah-like illness. Integrated One Health surveillance across wildlife, humans, and environment is critical for detection and prevention of emerging bat-borne viral threats.

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EVALUATING THE OPERATIONAL PERFORMANCE AND UTILITY OF THE D-MOSS (DENGUE FORECASTING MODEL SATELLITE-BASED SYSTEM) IN VIETNAM AND MALAYSIA

Amy M. Campbell[1], Felipe Colón-González[2], Do K. Quoc[3], Nguyen H. Tuan[4], Nguyen T. Dong[5], Tran T. Trang[6], Lokman H B Sulaiman[7], Shew F. Wong[7], Barbara Hofmann[8], Gina Tsarouchi[8], Quillon Harpham[9], Vu Sinh Nam[4], **Oliver Brady**[1]

[1]London School of Hygiene and Tropical Medicine, London, United Kingdom, [2]Wellcome Trust, London, United Kingdom, [3]Pasteur Institute, Ho Chi Minh City, Vietnam, [4]National Institute of Hygiene and Epidemiology, Hanoi, Vietnam, [5]Pasteur Institute, Nha Trang, Vietnam, [6]Tay Nguyen Institute of Hygiene and Epidemiology, Dak Lak, Vietnam, [7]IMU University, Kuala Lumpur, Malaysia, [8]HR Wallingford, Oxfordshire, United Kingdom, [9]HR Wallingford, London, United Kingdom

Despite substantial advances in dengue forecasting, few models have transitioned into sustained operational use. This requires managing data pipeline interruptions, repeated real-time estimation, and demonstrating value across outbreak planning and response scenarios. D-MOSS (Dengue forecasting Model Satellite-based System), a climate-based statistical model co-developed with the Vietnamese Ministry of Health, has operated nationally in Vietnam since June 2019, delivering near-real-time probabilistic forecasts across all 63 provinces. We present a prospective evaluation of D-MOSS under operational conditions, combining statistical performance with decision-relevant utility. Forecast skill was assessed against null seasonal baselines across incidence magnitude, temporal trajectories, and peak timing, while utility was evaluated via probabilistic classification of outbreak threshold exceedance across four policy scenarios. D-MOSS outperformed baseline models across most metrics, with highest accuracy at one-month lead times but sustained skill up to six months and greatest value-added at longer (4-6 month) horizons. Spatial variation was evident, with larger absolute errors in high-incidence central and southern provinces but greater relative gains over baselines. Anomalously early transmission in 2022 challenged peak timing predictions. Classification accuracy ranged from 0.83-0.94 depending on probability thresholds, with heterogeneous trade-offs between false alarms and missed outbreaks and spatial clustering of errors. To bridge the gap between forecast generation and use, stakeholder workshops in Vietnam and Malaysia engaged public

health practitioners to interpret forecast uncertainty, define acceptable risk thresholds, and integrate outputs into operational dengue control protocols. These informed co-developed decision frameworks linking probabilistic forecasts to staged response actions. Together, these findings demonstrate robust real-world performance while underscoring the need to embed forecasting within decision-making systems to realise operational value.

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THE THREAT OF ANTIMICROBIAL-RESISTANT TYPHOID: SURVEILLANCE CAPACITY AND VACCINATION RECOMMENDATIONS IN THE WHO EASTERN MEDITERRANEAN REGION

Yesser Sebeh[1], Ashley Tate Longley[2], Muhammad Tayyab[3], Nandini Sreenivasan[2], Kashmira Date[2], Eric Daniel Mintz[2], Evans Buliva[3], Lucy Breakwell Nagle[2]

[1]Consultant to the CDC Foundation, Atlanta, GA, United States, [2]Centers for Disease Control and Prevention, Atlanta, GA, United States, [3]World Health Organization Regional Office for the Eastern Mediterranean, Cairo, Egypt

Annually, typhoid fever causes up to 9.23 million cases and 163,000 deaths worldwide. The World Health Organization (WHO) Eastern Mediterranean Region (EMR) faces a high estimated incidence of typhoid fever and an increasing threat of antimicrobial-resistant (AMR) Salmonella Typhi, including extensively drug-resistant (XDR) strains. To inform regional prevention efforts, we assessed existing typhoid surveillance capacity and vaccination policies. In 2021, cross-sectional structured electronic surveys on typhoid surveillance and vaccination policies were distributed to surveillance and immunization officers in 22 EMR countries. We received completed surveillance surveys from 17 (77%) countries; 13 (59%) completed the vaccination policies survey. Fifteen (88%) of the 17 countries routinely collected typhoid surveillance data, and 11 (65%) used standard case definitions. While nine (60%) countries implemented facility-based, laboratory confirmed surveillance, only five (29%) predominantly used blood culture, the WHO-recommended standard. Seven (41%) predominantly relied on Widal tests (low sensitivity/specificity). Routine antimicrobial susceptibility testing (AST) was performed on S. Typhi isolates in seven reporting countries, and AMR was reported in five (Iraq, Iran, Oman, Pakistan, Yemen), with resistance to first-line antimicrobials and second-line antimicrobials such as fluoroquinolones and cephalosporins. Only two countries recommended typhoid vaccination: Pakistan introduced typhoid conjugate vaccines (TCV) in 2019 following an XDR outbreak, and Iraq recommends unconjugated vaccines for high-risk groups. No other surveyed countries planned to introduce TCV, citing insufficient disease burden data, perceived low incidence, and vaccine cost. Ultimately, typhoid surveillance and AST capacity remain weak in the region, resulting in limited reliable data for TCV introduction. Strengthening blood culture-based surveillance is critical to monitor AMR trends, understand national burden and risk, and inform prevention and vaccination strategies, especially in resource limited settings.

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INFORMING A GLOBAL INVESTMENT CASE FOR VACCINE-PREVENTABLE DISEASE SURVEILLANCE: A CONCEPTUAL FRAMEWORK

So Yoon Sim[1], Raymond CW Hutubessy[1], Carolina Danovaro[1], Jurjen Van der Schans[2], Maarten Postma[2]

[1]World Health Organization, Geneva, Switzerland, [2]University Medical Center Groningen, Groningen, Netherlands

Vaccine-preventable disease (VPD) surveillance is essential for monitoring epidemiological trends, optimizing immunization programs, detecting and responding to outbreaks, and tracking progress towards disease control, elimination, and eradication goals. However, surveillance systems are often fragmented, under-resourced, and designed around poorly defined use cases. The health and economic value of surveillance remains insufficiently evidenced, leading to underinvestment in the context of shrinking fiscal space. A structured framework is needed to define and quantify the value of strengthened surveillance for various VPDs. We develop a conceptual economic modeling framework using a multi-phase approach. First, a rapid scoping review systematically synthesizes available evidence on health and economic impact of VPD surveillance. Second, a structured conceptual modelling process, is applied to engage with relevant stakeholders to define key questions, identify data needs, and specify model scope and structure for future research. The framework delineates surveillance functions across the immunization value chain and identifies key cost drivers and impact pathways. It links system functions and interventions (e.g. laboratory and diagnostics strengthening, surveillance capacity building, system optimization, etc.) to improvements in surveillance quality indicators and downstream public health actions such as outbreak detection, vaccination targeting, and optimized vaccination policy recommendations. They are connected to health and societal outcomes, including cases and deaths averted, healthcare costs saved, and macroeconomic effects. Key methodological and evidence gaps are identified. This framework supports quantifying the value of VPD surveillance strengthening and informs investment and financing decisions, advancing system-level economic evaluation for immunization and health systems.

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EVALUATION OF *PLASMODIUM FALCIPARUM* SUSCEPTIBILITY TO ARTEMISININ USING THE RING-STAGE SURVIVAL ASSAY AND GENOMIC ANALYSIS

Agnes Chelangat Cheruiyot[1], Edwin Mwakio[1], Farid Abdi[1], Gladys Chemwor[1], Dennis Juma[1], Benjamin Opot[1], Raphael Okoth[1], Maurine Atieno Mwalo[2], Risper Nyaboke Maisiba[3], Redempta Yeda[1], Luis Powsang[1], Hoseah Akala[4]

[1]Walter Reed Army Institute of Research (WRAIR-Africa), Kenya Medical Research Institute (KEMRI), Kisumu, Kenya, [2]USAMRD-K, Kisumu, Kenya, [3]Kisumu, Kenya, [4]USAMRU-K, Kisumu, Kenya

Artemisinin combined therapies (ACTs) are approved current frontline antimalarials for treatment of malaria in Kenya. However, confirmed reports of artemisinin resistance in Southeast Asia pose a significant threat. This study evaluated susceptibility of *Plasmodium falciparum* using Ring stage survival assay (RSA) and Malaria SYBR Green I method: Objectives 1. Determine the percentage survival of parasites in field isolates treated with 700nm dihydroartemisinin. 2. Determine susceptibility of six antimalarials. 3. Determine the drug makers and correlate with susceptibility. A 2-3 mL of blood samples were collected in an approved malaria surveillance study. Subsets were treated with 700nm dihydroartemisinin while controls treated with dimethyl sulfoxide for 6 hrs. After the drug was washed and recultured for 66hrs. Upon termination microscopy was done to determine parasite percentage survival. Chloroquine (CQ), quinine (QN), dihydroartemisinin (DHA), piperaquine (PPQ), artesunate (AS) and mefloquine (MQ) susceptibility with Pfk*13*, *Pfmdr1*, and *Pfcrt* gene analysis. Of 73 isolates, 24 analyzed by ring-stage survival assay (RSA), 18 samples had survival rates >1%. Overall, 28/73 (38.4%) isolates harbored Pfk13 ART mutations. Median IC_{50} values (IQR) for chloroquine (CQ), quinine (QN), dihydroartemisinin (DHA), piperaquine (PPQ), artesunate (AS), and mefloquine (MQ) were 10.95 nM (9.35–15.26), 10.83 nM (6.84–25.55), 1.71 nM (0.83–2.12), 55.96 nM (40.81–74.06), 0.75 nM (0.27–3.43), and 8.88 nM (6.58–15.54). Among RSA-tested isolates, 20/24 harboured Pfmdr*1* 184 mutation. Four isolates had *CVIET* haplotype with higher CQ median IC_{50} compared with D6 strain (P=0.0001). In conclusion, Identification of *Pfk13* mutations in Kenya using RSA assay makes it a key method in surveillance of ACTs. A strategy of multiple first line therapy is recommended to prevent emergence of drug resistance.

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MULTIDIMENSIONAL BULK SEGREGANT ANALYSIS AND GENETIC ENGINEERING REVEAL THE PLEIOTROPIC CONTRIBUTION OF THE AUTOPHAGY GENE PFATG18 TO THE POLYGENIC LANDSCAPE OF MULTIDRUG RESISTANCE IN *PLAMODIUM FALCIPARUM*

Nirjhar Bhattacharyya[1], Lisa Checkley Needham[1], Douglas Shoue[2], Malaria Crosses Collaboration (MCC)[3], Angana Mukherjee[1], Michael Ferdig[4]

[1]University of Notre Dame, Notre Dame, IN, United States, [2]Univ of Notre Dame, Notre Dame, IN, United States, [4]Univ of Notre Dame, Dept of Biology, Notre Dame, IN, United States

Artemisinin-based combination therapies (ACTs) are threatened by treatment failure driven by resistance to artemisinin (ART-R), alongside multidrug resistance to chloroquine (CQ) and partner drugs like mefloquine (MFQ), piperaquine (PPQ) and lumefantrine (LUM). Major effectors include *pfkelch13* (ART-R), pfcrt (CQ resistance and with *pfpm2/3* amplification, PPQ resistance), and *pfmdr2* copy-number variation (MFQ resistance). However, to define the broader polygenic architecture of multidrug resistance, we performed dose-dependent multidimensional bulk segregant analysis (BSA) using a genetic cross between multidrug-resistant Cambodian KH004 (KEL1/PLA1; PfKelch13^{C580Y}, Dd2-like PfCRT, 4 copies of *pfpm2/3*) and sensitive Malawi (Mal31) parent clones. Recombinant progeny pools under distinct drug pressures—700 nM DHA (6 h); 50 nM, 100 nM and 250 nM CQ; and 31 nM and 62 nM PPQ—identified major loci *pfkelch13* (DHA), *pfcrt* and *pfaat1* (CQ), and *pfcrt* with *pfpm2/3* CNVs (PPQ), along with secondary signals including the autophagy gene *pfatg18* (Chr 10). Prior GWAS associated PfATG18^{T38I} with ART-R (patient clearance half-life) and CQ IC_{50}, while QTL mapping linked *pfatg18* to MFQ, LUM, and PPQ responses. Analysis of over 25,000 global isolates (1993-2019) from MalariaGEN showed elevated T38I frequency in Southeast Asia compared to Africa. Functional perturbation of PfATG18 using rapamycin-induced knock-sideways (protein-level mislocalization from the food vacuole to the cytosol) in an NF54^{Kelch13C580Y} background caused growth defects and reduced DHA ring stage survival (RSA dropped from 15% to 3%), decreased MFQ and CQ IC_{50}; and increased PPQ IC_{50}. Together, these results demonstrate that multidimensional BSA with targeted PfATG18 perturbation reveals its pleiotropic role linking digestive vacuole biology to multidrug resistance.

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A *PLASMODIUM FALCIPARUM* PX1 HAPLOTYPE IS ASSOCIATED WITH REDUCED SUSCEPTIBILITY TO ARTEMISININ AND LUMEFANTRINE

Christopher Bower-Lepts[1], Arvind Jangra[1], Sachie Kanatani[2], Abhai K. Tripathi[2], Godfree Mlambo[2], Alejandro McCotter Gonzalez[1], Linda Romano[1], Stephen Orena[3], Martin Okitwi[3], Karamoko Niaré[4], Philip Rosenthal[5], Melissa Conrad[2], Photini Sinnis[2], Sachel Mok[1]

[1]Columbia University Irving Medical Center, New York, NY, United States, [2]Johns Hopkins University, Baltimore, MD, United States, [3]Infectious Diseases Research Collaboration, Kampala, Uganda, [4]Department of Pathology and Laboratory Medicine, Brown University, RI, United States, [5]University of California San Francisco, Mill Valley, CA, United States

Effective malaria control depends on the sustained efficacy of frontline antimalarials, particularly artemether–lumefantrine (AL), the most widely used combination therapy in Africa. However, the recent emergence of artemisinin partial resistance and reduced lumefantrine (LUM) susceptibility in East Africa undermines disease control efforts. Robust genetic markers of AL resistance are vital for molecular surveillance but remain elusive. To elucidate the genetic basis of reduced AL susceptibility, we performed a *Plasmodium falciparum* genetic cross between the drug-sensitive NF54 strain and a Ugandan K13 mutant, dihydroartemisinin (DHA) resistant isolate with reduced LUM sensitivity. From targeted nanopore sequencing applied on bulk progeny pools exposed to DHA, LUM or mefloquine (MEF) pressure, we observed that DHA selected for distinct haplotypic signatures compared to LUM or MEF, suggesting differential modes of drug action and resistance. In drug-treated progeny pools and across ~390 recombinant progeny clones, amplicon sequencing analysis identified the *px1* gene, encoding a phosphoinositide-binding protein, as the strongest correlate of reduced DHA, LUM or MEF sensitivity. In addition, the PX1 PIN haplotype (L1222P/M1701I/D1705N) observed in our cross was among the most highly enriched markers following DHA or LUM selection of a mixed pool of 18 genetically diverse Ugandan clinical isolates subjected to various drug pressures. This finding indicates the recurrent selection of the PX1 PIN haplotype across diverse parasite backgrounds. *In vitro* dose-response assays of culture-adapted clinical isolates confirmed that PX1 PIN was associated with reduced LUM and DHA susceptibility even in the absence of K13 mutations. Competitive growth assays revealed that this haplotype was associated with an enhanced parasite asexual replication rate that may explain the increased prevalence of PIN mutations in Uganda over the last two decades. These findings suggest *px1* as a robust marker of reduced AL susceptibility and support its inclusion in genomic surveillance strategies to monitor emerging multidrug resistance in Africa.

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PFK13-ASSOCIATED ARTEMISININ RESISTANCE SLOWS DRUG ACTIVATION AND ENHANCES ANTIOXIDANT DEFENCE, WHICH CAN BE OVERCOME WITH SULFORAPHANE

Carlo Giannangelo[1], Ghizal Siddiqui[1], Natalie A. Counihan[2], Yunyang Zhou[1], Annie Roys[1], Amanda De Paoli[1], Bethany M. Anderson[3], Matthew P. Challis[1], Lee M. Yeoh[4], Stuart A. Ralph[3], Laura E. Edgington-Mitchell[3], Tania F. de Koning-Ward[2], Darren J. Creek[1]

[1]Monash University, Parkville, Australia, [2]Deakin University, Waurn Ponds, Australia, [3]The University of Melbourne, Parkville, Australia, [4]The University of Melbourne, Melbourne, Australia

Artemisinin resistance is globally prevalent, including in Africa. This raises concerns about the future control of malaria and highlights the need to better understand the cellular mechanisms underpinning artemisinin resistance. In *Plasmodium falciparum*, artemisinin resistance is primarily attributed to mutations in the PfKelch13 (PfK13) gene. In this study, we performed proteomic analysis on a range of sensitive and artemisinin-resistant parasites (both laboratory-generated and field isolates), revealing specific dysregulation of PfK13 protein abundance, independent of PfK13 mutation or parasite line, in early ring-stage parasites known to be critical in resistance. Decreased PfK13 levels were linked to impaired haemoglobin digestion, decreased free haem levels, and consequently, decreased artemisinin activation, which was measured directly using a LC-MS-based assay. Artemisinin resistant parasites also exhibited elevated thiol levels, including the main cellular antioxidant, glutathione, and its precursor, gamma glutamyl-cysteine, suggesting that resistant parasites possess an elevated antioxidant capacity. We hypothesised that targeting parasite antioxidant capacity with redox-modifiers would re-sensitise resistant parasites to artemisinin and identified the nutraceutical sulforaphane as a promising candidate that rapidly reacts with parasite glutathione and alters parasite redox status. Importantly, sulforaphane potentiated artemisinin activity in an RSA format *in vitro* and in a *P. berghei* model of artemisinin resistance *in vivo*, offering a potential strategy to overcome resistance. Our findings provide critical insights into the molecular mechanisms of artemisinin resistance and suggest novel therapeutic interventions to restore drug sensitivity.

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DORMANCY AND SURVIVAL OF *PLAMODIUM FALCIPARUM* BLOOD-STAGE PARASITES EXPOSED TO ARTEMISININ-BASED COMBINATION THERAPY (ACT)

Sasha Abielmona[1], Brian Yuzhe Xi[2], Jianbing Mu[3], Thomas E. Wellems[4], **Juliana M. Sa**[5]

[1]Rockville, MD, United States, [2]North Potomac, MD, United States, [3]National Institutes of Health, Rockville, MD, United States, [4]NIAID DIR, Gaithersburg, MD, United States, [5]NIAID, Rockville, MD, United States

Artemisinin-based combination therapies (ACTs) are the cornerstone of treatment for uncomplicated *Plamodium falciparum* malaria, yet their efficacy can be compromised by dormant blood-stage parasites that survive artemisinin exposure and later drive recrudescence. While artemisinin-induced dormancy has been proposed as a contributing mechanism to recrudescence, the role of partner drugs in the recovery of dormant parasites, independent of resistance-conferring mutations, has yet to be established. To investigate how partner drugs impact parasite dormancy and recrudescence, we compared outcomes in an antimalarial-sensitive NF54 parasite line following 3-daily in vitro monotherapy or combination therapy treatments with dihydroartemisinin (DHA), amodiaquine, piperaquine, and chloroquine. We hypothesized that 4-aminoquinoline drugs may impact persister survival by targeting parasite-derived heme and possibly reducing DHA activation. Recrudescence was monitored longitudinally by microscopic examination of thin blood smears. Our findings showed persistence followed by survival in both monotherapy and combination therapy. Recrudescence times across conditions ranged from day 15 to day 30 post-treatment and differed in persister morphology composition. This suggests that, under these conditions, partner drugs do not effectively eliminate DHA-induced persisters and may even induce dormancy themselves when administered alone. These experiments were conducted in vitro in the absence of an immune response, and although partner drugs achieve higher, more sustained plasma concentrations in vivo, recrudescence still occurs in approximately 5% of cases. These findings highlight the importance of considering parasite dormancy when evaluating ACT efficacy, as selection of persistent parasites over three days of treatment may result in populations that are resilient to various stressors, including other antimalarials.

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LUMEFANTRINE MOLECULAR RESOLUTION IMAGING AND PULSE-DRUG *PLASMODIUM FALCIPARUM* BIOASSAYS FOR HEME CRYSTAL INHIBITION COMPARED TO OTHER QUINOLINES AND THE HEME-ARTEMISININ ADDUCT

Huan-Jui Lee[1], Sandra Massih[2], Mahnoor Abdullah[2], Hariharan Annadurai[1], Jeffrey Rimer[1], **David J. Sullivan**[2], Peter G. Vekilov[3]

[1]University of Houston, Houston, TX, United States, [2]John Hopkins Univ, Baltimore, MD, United States, [3]Houston, TX, United States

Heme crystallization is central to malaria parasites' erythrocytic-stage survival, yet how clinically important antimalarial drugs interact to modulate this process at the molecular level remains unclear. Heme crystallization in *P. falciparum* is a dynamic-fluid to crystal multi-step process with steps, kinks, terraces as well as nucleation steps providing possible antagonistic or synergistic drug interactions. Previous work indicated a quinoline-like second mechanism of action-heme crystal inhibition for the artemisinin heme-drug adduct with low nanomolar IC50s, similar to other quinolines. Here we investigate the interactions between lumefantrine (LF), one of the popular components of current combination therapies, and hematin crystallization employing stage-resolved parasite assays and multiscale in vitro approaches compared to other quinolines and the heme-artemisinin adduct metabolite. Pulse LF suppresses *Plasmodium falciparum* during both ring and trophozoite stages. Under biomimetic conditions, LF markedly reduces β-hematin growth rates. High-resolution atomic force microscopy captures individual LF molecules binding directly to crystal steps, mechanically constraining step motion through a previously unreported step-capping mechanism. This localized inhibition propagates to the mesoscale, producing step broadening, reduced inter-step spacing, and causing surface kinetic roughening. In contrast to its strong inhibition of crystal growth, LF enhances hematin nucleation by promoting the formation of mesoscopic hematin-rich clusters, as detected by dynamic light scattering and Brownian microscopy. Attachment of the nucleated crystallites strains big crystal surfaces and thus impedes step growth. These findings define a non-classical, multiscale framework for LF-mediated inhibition of hematin crystallization and provide direct molecular insight into an important antimalarial mode of action. This work contributes to understanding interactions for triple artemisinin combination therapy on hemozoin.

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EFFICACY OF ARTEMETHER-LUMEFANTRINE AGAINST *PLASMODIUM FALCIPARUM* IN FRENCH GUIANA: A RETROSPECTIVE STUDY

Celia Florimond[1], Christophe Heugas[1], Richard Naldjinan[2], Yassamine Lazrek[1], Luana Mathieu[1], Beatrice Volney[1], Magalie Demar[2], Felix Djossou[2], Lise Musset[1]

[1]Pasteur Institute in French Guiana, Cayenne, French Guiana, [2]CHU Guyane, Cayenne, French Guiana

Artemether-Lumefantrine (AL), the most used artemisinin-combination based therapy (ACT) against malaria at *Plasmodium falciparum*, depicts globally a high efficacy worldwide. However, critical rates of treatment failure (>10%) have been reported in some African countries. Although this ACT has been the first-line treatment for uncomplicated malaria in French Guiana (low-transmission area) since 2007, data on AL efficacy remain limited for the Guiana Shield. Here, we describe a retrospective evaluation of AL therapeutic efficacy in patients routinely treated for *P. falciparum* and monitored in French Guiana between 2009 and 2023. A panel of 294 patients treated with AL were analyzed. Three-quarters (n=220/294) were excluded from the subsequent study of whom 63.6% (n=140/220) were lost to follow-up. Treatment response was evaluated in the remaining 74 patients (54 infected in Guiana Shield countries, 5 in African countries, and 15 unknown) using WHO protocol criteria. Fifty-seven patients presented adequate clinical and parasitological responses. Cases with recurrent parasitemia were investigated (plasma drug concentration, genotyping, phenotyping) to determine treatment failure causes. Treatment failure occurred in 24.3% of patients [95% CI: 14.5 - 34.0%], n=18/74), with eight cases (44.4%, n=8/18) associated with subtherapeutic drug levels. For 2 cases, reinfection or recrudescence could not be confirmed. The remaining 8 cases occurred mainly between 2009 and 2015, of these 25% were classified as late parasitological failure (n=2/8) and 75% as late clinical failure after at least 14 days (n=6/8). In most cases, parasites originated from the Guiana Shield (n=7/8), no partial artemisinin resistance was observed, and all isolates were sensitive to lumefantrine *in vitro*. One case, originating from Africa, showed slight tolerance to lumefantrine *in vitro*. Despite the limited sample size and the long study period, these findings showed a high rate (12.5%, n=8/64) of treatment failure potentially associated with resistance under AL in French Guiana and emphasize the importance of treatment adherence and systematic follow-up.

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UNVEILING THE PATHOGENESIS OF CHRONIC DIGESTIVE CHAGAS DISEASE: CONNECTING IMMUNE SIGNALING AND FIBROSIS

Erica Silberstein[1], Syros Karaiskos[2], Nikki Tirrell[2], Charles C. Chung[2], Supriya Kumar[1], Jung-Sun Cho[1], Alain Debrabant[1]

[1]FDA/CBER/OBRR, Silver Spring, MD, United States, [2]FDA/CBER/HIVE, Silver Spring, MD, United States

Transmitted primarily through insect vectors and blood transfusion, the protozoan parasite *Trypanosoma cruzi* represents a significant risk to the blood supply in countries where donor testing is not routinely implemented. In its chronic phase, the infection can cause Chagasic megacolon, characterized by progressive inflammation, excessive extracellular matrix

(ECM) protein deposition, and neuronal impairment leading to colonic dysfunction. Although parasite persistence is thought to drive this pathology, the molecular factors orchestrating the process remain poorly understood. To address this gap, we employed single-cell transcriptomic profiling in a murine model of Chagas disease to map the cellular landscape of the chronically infected colon. Cells from infected and naïve mice were processed using the BD Rhapsody single-cell analysis system followed by Illumina sequencing. Our results revealed significant immune infiltration in the colonic lamina propria, dominated by T cells and macrophages, a finding validated by flow cytometry and RT-qPCR of selected genes. Computational analysis using CellChat predicted two interconnected networks that may mediate disease mechanisms. The first network involved the CCL chemokine pathway, with the CCL5-CCR5 axis likely playing a key role in regulating interactions between CD8+ T cells and macrophages. The second network pointed to a significant disruption of colonic homeostasis, highlighting fibroblast-smooth muscle cell crosstalk identified as a central feature. This interaction, mediated by periostin and CXCL immune pathways, was predicted to drive increased collagen deposition, ECM remodeling and smooth muscle cell dysfunction —hallmarks of digestive Chagas disease (DCD) — a finding supported by histological analysis. In summary, our integrated analysis demonstrates that the pathogenesis of chronic DCD involves a complex interplay between the immune response and tissue remodeling. The identified CCL5-CCR5 and periostin/CXCL axes provide a foundation for developing novel disease biomarkers and targeted therapies aimed at preserving gut function in patients with CD.

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CHAGAS DISEASE RISK FACTORS AMONG A LOW-INCOME POPULATION AND EVIDENCE OF CHAGAS-ASSOCIATED CARDIOMYOPATHY, IN THE DC METROPOLITAN AREA

Alisson Melissa Baya[1], Rachel Marcus[2], Amira Roess[1]

[1]George Mason University, Fairfax, VA, United States, [2]LASOCHA (Latin American Society Of Chagas), Washington, DC, United States

Chagas disease, caused by *Trypanosoma cruzi*, is a neglected tropical disease endemic to Latin America and an emerging global health concern driven by migration. In the United States, an estimated 288,000 individuals are infected, yet fewer than 1% are diagnosed, and most clinicians do not routinely test for it. The aim of this study is to identify risk factors for Chagas seropositivity among a Latino immigrant population in the Washington Metropolitan area. The Latin American Society for Chagas (LASOCHA) screened individuals at community health fairs from September 2018 to September 2025. Participants completed a survey capturing demographic information and history of extended residence in endemic areas. Seropositivity was defined as testing positive on both the Chagas DetectTM Plus Rapid Test and a confirmatory serological test. Confirmed cases underwent electrocardiography (EKG). Multivariable logistic regression was used to assess risk factors for seropositivity and EKG abnormalities. Of 1,655 screened individuals (median age 46 years; 60% female), most originated from Bolivia (39%) or El Salvador (27%). Seroprevalence was 12.3% (95% CI: 10.8%-14.0%), with the highest rates among Bolivian-born participants (23.9%), followed by El Salvador (6.2%) and Guatemala (3.9%). Prevalence was similar by gender (females 12.0%, males 12.4%). Older age was significantly associated with seropositivity (OR = 1.05/year; $p < 0.001$); gender was not ($p = 0.56$). Among 62 seropositive individuals with EKG data, 50% had cardiac abnormalities consistent with Chagas-associated cardiomyopathy. Community-based screening identified a seroprevalence of 12.3%, with Bolivian-born participants disproportionately affected. Older age was a significant predictor of seropositivity, while gender was not. The high rate of EKG abnormalities underscores the need for integrated cardiac monitoring in targeted, high-risk screening programs.

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OBESITY-ASSOCIATED INFLAMMATION AND THERAPEUTIC FAILURE IN CUTANEOUS LEISHMANIASIS

Mauricio Nascimento[1], Augusto Carvalho[2], Marina Santana[3], Edgar M. Carvalho[4], **Lucas P. Carvalho**[5]

[1]Fiocruz, SALVADOR, Brazil, [2]Fiocruz, Salvador, Brazil, [3]Fiocruz-Bahia, Salvador, BA, Brazil, [4]Federal University of Bahia, Salvador, Brazil, [5]Federal University of Bahia, SALVADOR, Brazil

Cutaneous leishmaniasis (CL) patients have high inflammatory response that is associated with skin ulcer development. Obesity is a significant public health issue, and its prevalence is increasing in developing countries. Interestingly, obese CL patients present more severe disease and higher rates of therapeutic failure to pentavalent antimonial compared to lean CL patients. Hypercholesterolemia and hypertriglyceridemia have been associated with inflammatory clinical conditions, such as atherosclerosis. It is well established that increased cholesterol and LDL levels lead to foam cell formation, as well as activation of the NLRP3 inflammasome and production of IL-1β, a cytokine known to be directly involved in the immunopathogenesis of CL. Our data show that CL patients present hypercholesterolemia and hypertriglyceridemia, along with increased levels of apolipoproteins associated with LDL, chylomicrons, and triglycerides compared to healthy individuals. In addition, genes involved in LDL uptake, cholesterol efflux, and foam cell formation are upregulated in CL lesions and positively correlated. Functional assays using peripheral blood mononuclear cells from CL patients and macrophages infected with *L. braziliensis* show that in vitro treatment with cholesterol, HDL, and LDL induces an inflammatory response. Interestingly, macrophages treated with cholesterol exhibited higher infection rates during the early time points. Finally, we found that obese CL patients have higher levels of cholesterol, LDL, VLDL, triglycerides, APOE, and APOB100 compared to lean individuals with CL. Our data suggest that obesity and cholesterols are associated with inflammation and poor clinical outcome in CL.

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LOSS OF MYENTERIC GANGLION CELLS UNDERLIES CHAGASIC MEGACOLON

Sylvia S. Sanchez[1], Eduardo Ribas[1], Sneider Alexander Gutierrez Guarnizo[1], Mauricio Tapia Guzman[2], Yoselin Conde[2], Eric Moughames[3], Rony Colanzi Zeballos[4], Robert Bulat[3], Robert H. Gilman[1], Jackie Birkness-Gartman6[5]

[1]Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [2]Hospital Universitario Japones, Santa Cruz de la Sierra, Plurinational State of Bolivia, [3]Department of Gastroenterology and Hepatology, Johns Hopkins School of Medicine, Baltimore, MD, United States, [4]Universidad Catolica, Faculdad de Medicina, Santa Cruz, Plurinational State of Bolivia, [5]Department of Pathology, Johns Hopkins School of Medicine, Baltimore, MD, United States

Chagas disease, caused by *Trypanosoma cruzi*, can lead to megacolon, a chronic complication driven by enteric nervous system injury. Proposed mechanisms include chronic inflammation, immune-mediated damage, and alterations in neuronal populations across key colonic layers, including the mucosa, submucosa, and muscularis propria. However, the histopathologic and cellular basis of disease pathogenesis remain understudied. Full-thickness colonic tissues from 38 patients in Bolivia were analyzed and categorized as seronegative for Chagas disease without megacolon (C–M–, n=11), seropositive without megacolon (C+M–, n=5), and seropositive with megacolon (C+M+, n=22). Samples were obtained from three distinct regions of each resected specimen, referred to as proximal, middle, and distal relative to the surgical specimen. Samples were evaluated using hematoxylin and eosin staining and immunohistochemistry (IHC) for Hu C/D (ganglion cells) and CKIT (mast cells, interstitial cells of Cajal). Quantification was performed using digital image analysis, and groups were compared using nonparametric tests. Crypt architectural distortion increased with disease progression (p=0.021), while other histologic features were not significantly different. Mononuclear ganglionitis was more common in seropositive patients, particularly those with megacolon, but was not

specific to disease status. A significant decrease in ganglion cell density within the muscularis propria was observed, with reduced ganglion cells/mm (p=0.011) and ganglia/mm (p=0.028) from C–M– to C+M+ groups. In contrast, no significant differences were observed in submucosal ganglion cell or ganglia densities. Mast cell density was increased in the submucosa of megacolon patients, while eosinophils and interstitial cells of Cajal were unchanged. No amastigotes were detected via IHC. Chagasic megacolon is characterized by progressive loss of myenteric ganglion cells, supporting neuronal depletion, rather than widespread inflammation, as a central mechanism of disease pathogenesis and a target for future spatial transcriptomic studies.

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TRANS-SIALIDASE-DERIVED VIRUS-LIKE PARTICLE (VLP) VACCINE CANDIDATE TSP-5 ELICITS BALANCED TH1/TH2 IMMUNITY AND INHIBITS IN VITRO *TRYPANOSOMA CRUZI* INFECTION

Ugochukwu Oduwe[1], Shefa Tabassum[1], Giorgi Kenkebashvili[1], Edward Ayala[2], M.G. Finn[3], Alexandre Marques[1]

[1]University of Southern Mississippi, Hattiesburg, MS, United States, [2]Universidad Nacional Mayor de San Marcos, Lima, Peru, [3]Georgia Institute of technology, Atlanta, GA, United States

Chagas disease is a neglected tropical disease endemic to large regions of Latin America, with increasing cases reported in the United States, Europe, and other non-endemic regions. Over six million individuals are affected globally, with a substantial proportion at risk of developing Chronic Chagas Cardiomyopathy, contributing to significant morbidity and mortality. Cases remain underreported in the United States, where only eight states mandate reporting of suspected cases to health authorities. Treatment options are limited; benznidazole and nifurtimox demonstrate a parasitological cure rate of less than 20% in chronic patients, compounded by considerable adverse effects and systemic toxicity, underscoring the urgent need for an effective vaccine. *Trypanosoma cruzi*, the causative agent of Chagas disease, is auxotrophic for sialic acid and scavenges it from host glycoconjugates via the trans-sialidase enzyme, a process critical to immune evasion and host cell invasion. Using immunoinformatic tools, we predicted conserved trans-sialidase epitopes and synthesized a target peptide conjugated to Qβ-bacteriophage Virus-Like Particles (VLPs) to enhance immunogenicity. The vaccine candidate TSP-5 was used to immunize C57BL/6 mice via a prime-and-two-boost regimen (20 µg/dose). Sera and splenocytes were collected to assess humoral and cellular immune responses. IgG quantification and cytokine profiling (TNF-α, IFN-γ, IL-10, and IL-4) demonstrated significant Th1/Th2 responses versus controls ($P < 0.01$). In vitro inhibition assays, confirmed by confocal microscopy, revealed significant *T. cruzi* inhibition, correlating with robust IgG titers and anti-TS specificity, supporting this VLP-based candidate as a viable and immunologically rational vaccine strategy.

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RE-EVALUATING THE WHO PRAZIQUANTEL DOSE POLE: IMPACT OF BMI ON TREATMENT ACCURACY IN ADULTS ACROSS ENDEMIC SETTINGS FOR SCHISTOSOMIASIS

Paprika Berry[1], Pedro Henrique Gazzinelli-Guimaraes[2]

[1]George Washington University, Washington, DC, United States, [2]The George Washington University, Washington, DC, United States

Praziquantel is a cornerstone of preventive chemotherapy (PC) for schistosomiasis and a key component of the 2021-2030 roadmap to eliminate schistosomiasis as a public health problem in 50 endemic countries. In 2024, 83 million school-age children (SAC) and 17 million adults were treated, primarily through large-scale mass drug administration (MDA) using the WHO PZQ dose pole. However, recent studies report a high frequency of adult underdosing (<30 mg/kg) by the dose pole, largely driven by overweight and obesity, now prevalent in many endemic regions (10-70%). We recently performed a multi-country systematic review and meta-analysis to assess dosing accuracy by age and BMI using the current WHO dose pole. The study included 64,212 individuals from 21 countries. The WHO dose pole showed high accuracy in SAC, with <3% underdosed, independent of BMI. In contrast, over 28% of adults were underdosed, especially those with BMI >25, raising concerns about treatment failure and potential resistance. Therefore, this new study evaluated the performance of the PZQ dose pole in adults and proposed alternative dosing strategies to improve dosing accuracy. Modeling alternative dosing strategies, using data from 4,380 adults from two countries (Mozambique and South Africa), revealed that adding one PZQ tablet (600 mg) to the dose determined by the dose pole for adults (>16 years old) significantly improved dosing accuracy, reducing the frequency of underdosing from 23% to 2.4% and increasing the proportion receiving optimal dosing (40-60 mg/kg) from 24% to >70%. Importantly, >95% of individuals receiving higher doses (>60 mg/kg), with the addition of one tablet, remained within a safe range (60-80 mg/kg). These findings demonstrate that the current WHO dose pole is well-suited for pediatric populations but insufficient for adult treatment in the context of rising global BMI. We propose a simple, universal addition of one tablet for adults, to improve treatment efficacy and support schistosomiasis elimination efforts. Ongoing work aims to validate these findings across additional endemic settings and inform future WHO guideline updates.

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LONG-TERM EFFECTS OF TREATMENT WITH PRAZIQUANTEL ON GENITAL SYMPTOMS AND ABNORMALITIES IN WOMEN WITH AND WITHOUT *SCHISTOSOMA HAEMATOBIUM*

Eugenia Lee[1], Loyce Mhango[2], Jane Kagoli Maganga[2], Crispin Mukerebe[2], Humphrey Deogratias Mazigo[3], Govert Van Dam[4], Danielle de Jong[4], Pytsje Hoekstra[4], Paul Corstjens[4], Saidi Kapiga[2], Myung-Hee Lee[1], John Changalucha[2], Jennifer Downs[1]

[1]Weill Cornell Medical College, New York, NY, United States, [2]Mwanza Intervention Trials Unit, Mwanza, United Republic of Tanzania, [3]Catholic University of Health and Allied Sciences, Mwanza, United Republic of Tanzania, [4]Leiden University Medical Centre, Leiden, Netherlands

Over 56 million women and girls are affected by female genital schistosomiasis (FGS) caused by *Schistosoma haematobium*. Despite single or multi-dose treatment regimens with praziquantel, studies with up to 9-month follow-up indicate persistent post-treatment symptoms and gynecologic abnormalities. Little data exist on longer-term effects of praziquantel. We quantified symptoms and gynecologic findings in a cohort of women with and without *S. haematobium* in Tanzania who underwent intensive management across 7 visits over 12 months, with final long-term follow-up at median 24 months post-cohort completion. At each visit, women provided medical history, urine for microscopy, serum for schistosome circulating anodic antigen (CAA), and had gynecologic examinations with testing for sexually transmitted infections (STI). At long-term visit, we collected cervical images with digital colposcopes to quantify the frequency and distribution of abnormalities. Women received praziquantel when eggs or CAA were positive, doxycycline for C. trachomatis, ceftriaxone for *N. gonorrhoeae*, and metronidazole for *T. vaginalis*. Long-term follow-ups were completed by 189 women, of whom 97 had *S. haematobium* during the initial year with accompanying symptom and exam records. Using univariable mixed-effects logistic regression, reported genital warts/rash (past two months) was lower at long-term visit (OR 0.28 [0.13-0.58], p=0.001), as was abnormal discharge on exam (OR 0.23 [0.09-0.60], p=0.002). Both associations remained significant after adjusting for current STI infection (genital warts/rash: aOR 0.20 [0.08-0.47], p<0.001; abnormal discharge: aOR 0.23 [0.08, 0.66], p=0.007). There were no changes in reported post-coital bleeding, dyspareunia, or past-year infertility. Receipt of doxycycline was not associated with long-term symptoms or gynecologic findings. Ongoing formal colposcopy image grading will be reported at ASTMH 2026. These data bolster prior evidence that praziquantel, even with frequent testing and treatment, can partially but not completely relieve gynecologic symptoms and pathology in women with *S. haematobium*.

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IMPACT OF A COMMUNITY-WIDE TREATMENT PROGRAM ON SCHISTOSOMIASIS TRANSMISSION

Yvonne Ashong[1], Frank Twum Aboagye[2], Samuel Armoo[2], Dziedzom K. de Souza[1], Mike Osei-Atweneboana[2]

[1]Noguchi Memorial Institute for Medical Research, Accra, Ghana, [2]Water Research Institute, Council for Scientific and Industrial Research, Accra, Ghana

Africa contributes to 93% of the 250 million schistosomiasis cases worldwide, despite the successes achieved through praziquantel treatment against the disease. Just like many NTDs, developing and adopting improved control strategies has the potential to significantly reduce disease burden. This study explored the impact of a community-wide praziquantel treatment (CWT) on the transmission and control of schistosomiasis in three endemic communities in Ghana. Baseline parasitological assessments were conducted among community members aged ≥ 5 years, after which praziquantel (40 mg/kg) and albendazole (400 mg single dose) were administered to 3,860 individuals, representing 88.5% treatment coverage. Followup assessments were conducted at 2, 6, and 12 months posttreatment to determine changes in prevalence and intensity of both intestinal and urogenital schistosomiasis. Freshwater snails were also collected from water contact sites in the communities to assess the rate of transmission after treatment. At baseline, prevalence of *S. mansoni* was 41.6%, declining significantly to 21.0% at 2 months and remaining relatively stable at 23.4% at 6 months and 30.4% at 12 months. Similarly, *S. haematobium* prevalence decreased from 12.4% to 7.8% at 2 months, followed by slight increases to 2.1-12.9% at 6 months across communities and 3.5-17.8% at 12 months. Snail surveys revealed that both *Bulinus* and *Biomphalaria* species continued to shed humaninfective cercariae at all time points, although shedding was lowest at 2 months posttreatment. Using Sidakcorrected comparisons, significant differences were observed between human and snail infection prevalence for *S. mansoni,* while differences for *S. haematobium* varied across communities. Overall, the findings demonstrate that while CWT substantially reduces infection in the short term, reinfection occurs rapidly in this hightransmission setting. Expanded access to praziquantel is beneficial, but longterm control and progress toward elimination will require integrated approaches including snail control, environmental management, and improved access to clean water.

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SAFETY, TOLERABILITY, AND CLINICAL OUTCOMES FOLLOWING ORAL ADMINISTRATION OF TRICLABENDAZOLE IN PATIENTS (SIX YEARS OF AGE OR OLDER) WITH FASCIOLIASIS: A PHASE 4 STUDY

Nhu Thi Hoa[1], Essam Bedewy[2], Mustafa Kemal Celen[3], Rosa Maria Alfaro Carrasco[4], Damodaran Solai Elango[5], Nathalie Garzon Vanacker[6], Armin Weinzierl-Hinum[7], Rakesh Chugh[5], Richa Sharat Chandra[8], **Isabelle Meyer-Andrieux**[7]

[1]Oxford University Clinical Research Unit, Ho Chi Minh, Vietnam, [2]Alexandria University, Alexandria, Egypt, [3]Dicle University, Diyarbakır, Turkey, [4]Hospital Nacional Adolfo Guevara Velasco, Cusco, Peru, [5]Novartis Healthcare Private Limited, Hyderabad, India, [6]Novartis Pharma nv-sa, Vilvoorde, Belgium, [7]Novartis Pharma AG, Basel, Switzerland, [8]Novartis Pharmaceuticals Corporation, East Hanover, NJ, United States

Fascioliasis is a zoonotic infection caused by *Fasciola hepatica* and *Fasciola gigantica* that mainly infests goats, cattle and sheep, but can also cause disease in humans following ingestion of contaminated food or water. Human fascioliasis may present in the acute stage (also referred to as hepatic or invasive) or in the chronic stage (also referred to as biliary). The acute phase lasts 3-5 months and is associated with migration of flukes into the bile ducts. In the chronic phase, adult flukes reside in the bile ducts, causing inflammation and hyperplasia of the epithelium, and together with the physical presence of the parasite, may lead to biliary obstruction. Triclabendazole is the only treatment for fascioliasis recommended by the World Health Organization (WHO). Egaten™ (triclabendazole) was approved as a single dose of 10 mg/kg for the treatment of fascioliasis in patients aged ≥6 years. It was approved in the US (February 2019) at a regimen of two 10 mg/kg doses given 12 hours apart. This Phase 4 study (NCT04230148) is being conducted as a post-marketing commitment study for US FDA. It is a multi-center, open-label, non-comparative, single-arm study and has enrolled 301 patients from 10 sites across Vietnam, Turkey, Egypt, Peru and Colombia. The primary objective of the study is to evaluate the safety and tolerability of the two-dose regimen. The secondary objectives are to evaluate the clinical outcomes in both acute and chronic fascioliasis patients including clinical response at Day 10, and clinical cure at Day 30, Day 60 and Day 90 as well as parasitological cure in chronic fascioliasis patients. The study has finished enrolment and a summary of the results will be presented at the ASTMH 2026 Annual Meeting.

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EFFECTS OF PRAZIQUANTEL TREATMENT ON GENETIC DIVERSITY AND POPULATION STRUCTURE OF *SCHISTOSOMA HAEMATOBIUM* POPULATIONS IN TWO NEIGHBORING ENDEMIC VILLAGES IN NORTHERN SENEGAL

Omar K. Sow[1], Pape N. Diagne[1], Bruno Senghor[2], Marie P.F. Diouf[3], Souleymane Doucoure[4], Babacar Faye[5], Oumar Gaye[5], Ronald Edward Blanton[6], Doudou Sow[1]

[1]Gaston Berger University, Saint-Louis, Senegal, [2]Immunophysiopathologie et Maladies Infectieuses, Institut Pasteur, Dakar, Senegal, [3]Iba Der Thiam University, Thies, Senegal, [4]Institut de Recherche pour le Développement (IRD), Dakar, Senegal, [5]Cheikh Anta Diop University, Dakar, Senegal, [6]Tulane University, New Orleans, LA, United States

Mass drug administration (MDA) with praziquantel (PZQ) has been implemented in Senegal since 2009 to control schistosomiasis. In high-transmission areas, where prevalence may reach 80%, at-risk populations can receive up to two treatment rounds annually. Assessing the impact of PZQ on parasite genetic diversity and population structure is essential to evaluate its long-term effectiveness. A longitudinal survey was conducted among 600 individuals from two endemic villages (Ndiatene and Ntiagar) in northern Senegal, combined with an epidemiological questionnaire. Participants positive at baseline were treated and reassessed after one month, while the full cohort was followed up at six months. Logistic regression was used to identify infection risk factors. Pooled Schistosoma haematobium eggs were genotyped using microsatellite markers and analyzed in R software. Genetic differentiation was estimated using Nei's GST and Jost's D. Baseline prevalence was 27%, and 9 of 162 treated individuals still excreted eggs after one month. At six months, prevalence reached 8.2%. Infection risk was strongly associated with age, with children under 15 disproportionately affected, whereas higher education levels were protective. Genetic differentiation among infrapopulations was moderate (Di = 0.192), slightly higher in Ndiatene (0.211) than in Ntiagar (0.172). Differentiation between villages was lower (Dc = 0.064), suggesting some gene flow despite a 3 km distance. Following treatment, infrapopulation differentiation decreased at one month (0.185) before rising again at six months (0.226), while Dc reached 0.081. Urogenital schistosomiasis remains a major public health issue in northern Senegal. Despite repeated MDA, praziquantel remains effective, although rapid reinfection persists. These findings highlight the need for strengthened surveillance, particularly to detect early signs of reduced drug susceptibility.

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INTEGRATING MASS DRUG ADMINISTRATION INTO PRIMARY HEALTH CARE FOR SCHISTOSOMIASIS AND SOIL-TRANSMITTED HELMINTHS IN WESTERN KENYA: A QUASI-EXPERIMENTAL STUDY ON FEASIBILITY, COVERAGE, AND COST-EFFECTIVENESS

Chitiavi Josphat Mmasi Juma[1], Irene Natalie Chami[2], Wyckliff Peter Omondi[3], Dollycate Njagi[4]

[1]Amref Health Africa, Nairobi, Kenya, [2]The END Fund, New York, NY, United States, [3]Ministry of Health, Nairobi, Kenya, [4]Dollycate Njagi, Nrb, Kenya

Mass drug administration (MDA) for schistosomiasis (SCH) and soil-transmitted helminths (STH) in Kenya has traditionally relied on vertical campaigns, which are donor-dependent, episodic, and often miss adults and out-of-school children. Integrating MDA into primary health care (PHC) offers a pathway to sustainability, yet evidence from real-world settings remains limited. A quasi-experimental controlled before-and-after study was conducted across 24 wards in four endemic counties (Bungoma, Kakamega, Vihiga, Trans Nzoia). The intervention arm (12 wards) integrated STH/SCH treatment into routine PHC services through health facilities and community health promoters (CHPs), while the control arm (12 wards) continued campaign-based MDA. Data were collected from 1,130 households, 24 health facilities, 24 key informant interviews, and 8 focus group discussions. Outcomes measured included coverage, feasibility, cost-effectiveness, and health system readiness. Survey-measured coverage was high in both models; 90% in PHC-integrated areas vs. 92% in campaign areas ($p>0.05$). Compliance was near universal (99% vs. 100%). Facility readiness scores improved from 4.42 to 8.18 in intervention sites (+85%) vs. 4.17 to 6.82 in control sites. PHC integration achieved 44% lower total costs (USD 75,503 vs. USD 133,798) and cost per person treated of USD 0.25 vs. USD 0.31. Community concerns about deworming side effects dropped from 17% to 6% in intervention areas. A key challenge was drug stock-outs in 83% of intervention facilities at endline, highlighting supply chain gaps. In conclusion, integrating STH/SCH treatment into PHC is feasible, achieves coverage comparable to campaigns, and strengthens health system readiness at substantially lower cost. This model aligns with Kenya's UHC agenda and offers a sustainable pathway for NTD elimination, provided supply chains are strengthened to support continuous, consumption-based resynchronization

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DECODING THE SYNDEMIC: HELMINTH POLYPARASITISM AND THE LIMITATIONS OF RESTRICTED-SPECTRUM MASS DRUG ADMINISTRATION IN GHANA

Caleb Kobina Danso-Coffie[1], Belinda Aculley[2], Eric Kyei-baafour[3], Sophia Eyia-Ampah[1], Jones Amo Amponsah[4], Emmanuel Boafo[1], Rhoda Yeboah[5], Philip O. Banahene[6], Helena Lamptey[7], Michael Fokuo Ofori[8], Daniel Oduro[9]

[1]Noguchi Memorial Institute for Medical Research, Accra, Ghana, [2]Accra, Ghana, [3]Legon-Accra, Ghana, [4]Noguchi Memorial Institute, Ghana, Accra, Ghana, [5]Rhoda Yeboah, Accra, AA, Ghana, [6]University of Ghana, Accra, Ghana, [7]Noguch Memorial Institute, Legon, AA, Ghana, [8]Noguchi Memorial Institute for Medical Research, Accra, United States, [9]UNIVERSITY OF GHANA, Accra, AA, Ghana

Helminth polyparasitism in sub-Saharan Africa remains a critical public health challenge. In Ghana, national Mass Drug Administration (MDA) protocols rely on the standard co-administration of praziquantel and albendazole, often overlooking co-endemic liver flukes and complex species interactions. This study utilized molecular tools and multivariate analysis to characterize the epidemiological patterns and hematological impact of *Schistosoma mansoni*, *Fasciola* spp., and *Ascaris* spp. in children (n=192) from the schistosome-endemic communities, Tomefa and Asutsuare, in Ghana during the 2021 post-pandemic disruption period. Samples were screened via PCR and gel electrophoresis; Principal Component Analysis (PCA) identified latent clinical phenotypes, while logistic regression modeled environmental drivers. Overall prevalence was 49.5%, dominated by *S. mansoni* (29.2%), followed by *Fasciola* spp. (27.6%), with 16.1% harboring triple-species infections. Logistic regression linked Tomefa residency (OR=4.7, P<0.0001) and river contact (OR=3.5, P=0.0029) to infection risk. PCA revealed a distinct high-burden phenotype in Tomefa, accounting for 77.4% of triple infections. PC2 identified a "fluke-driven" component where *S. mansoni* and neglected *Fasciola* spp. exhibited strong co-loading (0.73 and 0.61, respectively), suggesting shared environmental niches and a pharmacological "blind spot," as *Fasciola* remains untreated by the standard two-drug MDA regimen. PC5 captured a syndemic hematological signature (MCV: 0.69; MCH: 0.69), characterizing the microcytic hypochromic anemia prevalent in polyparasitized individuals. These findings demonstrate that current dual-therapy strategies leave a substantial "residual burden" (*Fasciola* infection) in endemic hotspots. The clustering of triple-species infections and associated anemia underscores the inadequacy of existing protocols. We advocate for geographically targeted, multi-drug interventions based on endemic pathogens, including fluke-specific treatment, to break morbidity cycles in multiplex transmission environments.

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INTEGRATING SCREENING FOR PARASITIC DISEASES IN ANTENATAL CARE AND VAGINAL SELF-SAMPLING FOR FEMALE GENITAL SCHISTOSOMIASIS DURING PREGNANCY IN BAGAMOYO DISTRICT, TANZANIA

Amy S. Sturt

VA Palo Alto Healthcare Systems, Palo Alto, CA, United States

Neglected tropical disease (NTD) surveillance, including *S. haematobium* and female genital schistosomiasis (FGS) screening, often relies on school-based control programs that exclude adult women. Antenatal care (ANC) services are widely utilized in sub-Saharan Africa and offer a platform to integrate parasite screening with routine care to facilitate earlier diagnosis and treatment. This pilot study aimed to quantify the burden and consequences of malaria and NTDs (including FGS), among pregnant women in Bagamoyo, Tanzania. A secondary aim evaluated the acceptability of integrating parasite screening into ANC services. Women were enrolled from two communities between February 2024 and March 2025. Genital self-swabs, fingerprick blood, urine, and stool samples were collected. The acceptability and feasibility of integrated screening and presumptive treatment were assessed quantitatively among participants and healthcare workers. In this pilot, schistosomiasis surveillance was successfully incorporated into routine workflows for 413 women at first ANC, 94.2% (389/413) of whom contributed acceptability data. The majority of participants reported that vaginal self-sampling was "easy" or "very easy" (82.1%), were comfortable performing it (99%), and were willing to self-sample again in the future (99%). The prevalence of active schistosomiasis, as measured by circulating anodic antigen (CAA), was low (7.7%, 32/413), and only one PCR-positive FGS case was detected. However, the younger age profile of ANC attendees (~40% under 25 years) aligns with those at risk for FGS, a group underrepresented in traditional surveillance. Screening revealed a 4-fold higher prevalence of *Plasmodium falciparum* infection using molecular methods compared to routine RDTs, demonstrating that ANC-based molecular surveillance can uncover a substantial burden of subpatent infections early in pregnancy, prior to initiation of intermittent preventative treatment. Although *Schistosoma* prevalence was low, integrated malaria and NTD screening within ANC is feasible and acceptable among pregnant women.

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FROM NEGLECT TO NATIONAL PRIORITY: LIBERIA'S FIRST NATIONAL POLICY ON UROGENITAL SCHISTOSOMIASIS (UGS) AND LESSONS FOR HEALTH SYSTEMS INTEGRATION

Anthony Kerkula Bettee[1], Darwosu Borbor Dahn[1], Nana Ama Tiwaa-Boateng[2], Karsor K. Kollie[2], Anna Wickenden[2], Ashley Preston Preston[3], Yael Velleman[3]

[1]Ministry of Health, Monrovia, Liberia, [2]Effect Hope, Markham, ON, Canada, [3]Unlimit Health, London, United Kingdom

Urogenital Schistosomiasis (UGS) remains one of the most neglected public health conditions affecting women and men in Africa. Despite its substantial health, social and economic consequences, it remains largely absent from national policy, financing and health systems planning, contributing to continued neglect. Liberia's UGS strategic framework was developed to reposition UGS as a national health priority into integrated policy and systems action. Prolonged UGS predisposes women and men to long-term complications, with an increased risk of (STIs), HIV and HPV, fertility issues (miscarriages, subfertility and infertility), mental health challenges including depression, with wider impacts on livelihoods, gender equity and social wellbeing. This abstract presents lessons from Liberia's development of the first national policy on UGS globally, launched by the MOH in February 2026. The experience shows how policy can act as a transformative lever to break cycles of neglect by creating visibility, clarifying responsibility, and enabling coordinated responses across the health system. The policy establishes integrated implementation across prevention, case management and community health platforms and across health systems functions including supply chain and health promotion of UGS from a largely invisible condition to a recognised national health priority. The process brought together departments within MOH, the Ministries of Education and Agriculture, the National WASH Commission, and partners. Women affected by FGS actively shaped the policy process, ensuring lived experience informed decision-making and strengthening relevance for implementation. By clarifying roles and strengthening collaboration across sectors and programmes, the policy created a foundation for coordinated action demonstrating how policy can shift fragmented efforts towards coordinated national action. This experience provides lessons for integrating UGS within national health systems. Liberia's experience offers an important example of how country leadership and policy reform can reposition UGS from a neglected to a priority action.

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CHARACTERISTICS OF CHILDREN PRESENTING WITH TRACHOMATOUS TRICHIASIS AT SURGICAL CAMPS IN SOUTH SUDAN

Pallavi A. Kache[1], Madison L. Bearden[1], William Aguer[2], Reida Konga[2], Stephen O. Ohure[2], Nicholas A. Presley[1], Albino W. Nyibong[3], Yak Yak Bol[3], E. Kelly Callahan[1], Angelia Sanders[1], Scott D. Nash[1]

[1]The Carter Center, Atlanta, GA, United States, [2]The Carter Center, Juba, South Sudan, [3]Ministry of Health, Republic of South Sudan, Juba, South Sudan

Repeated *Chlamydia trachomatis* infection can cause conjunctival scarring and progression to trachomatous trichiasis (TT), wherein eyelashes abrade the cornea and may lead to blindness. Pediatric TT is rare globally and poorly characterized clinically and within surveillance systems. In trachoma hyperendemic counties in South Sudan, the Trachoma Control Program has increasingly registered suspected pediatric TT during TT surgical outreach camps and aimed to characterize cases among children attending these camps. In July 2025 and February 2026, children ages 1-14 years presenting with suspected TT to two surgical camps in Pibor County were enrolled in a cross-sectional descriptive study. Participants were assessed with a questionnaire capturing demographics and clinical history, followed by trachoma grading conducted by a certified grader. Grading assessed standard trachoma signs—trachomatous inflammation-follicular (TF), trachomatous inflammation-intense (TI), and trachomatous scarring (TS)—and TT severity and corneal opacity (CO). Children ages 1-14 years comprised 4.1% (239/5,702) of surgical camp attendees. Among all children, 32 (13%; 32/239; ages 3-14 years) had confirmed TT. Major TT (>5 lashes touching the globe) was present in 30/32 (94%) children, and 29/32 (91%) had bilateral TT. CO was observed in 21/32 (66%) children. Trachoma signs were common with TF in 21/32 (66%) children, TI in 21/32 (66%) children, and TS in 25/32 (78%) children. In 4 children, eyelid eversion was not possible due to severe entropion. Results suggest an under-documented burden of pediatric TT in this setting, with bilateral involvement, scarring, and CO. Observed children were either operated on at the Pibor surgical camp or referred to a Ministry of Health eye clinic in Juba. Ongoing work includes serologic testing and photo grading to characterize patients' exposure history and disease progression. The findings underscore an urgent need to resume mass drug administration in Pibor County, which was last treated in 2007. Population-level data are needed to clarify underlying pediatric TT epidemiology and guide further interventions.

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SAFETY, EFFECTIVENESS AND COST-EFFECTIVENESS OF LEVERAGING SEASONAL MALARIA CHEMOPREVENTION PLATFORM FOR CODELIVERY OF ALBENDAZOLE AND PRAZIQUANTEL AMONG SCHOOL-AGED CHILDREN IN GHANA

Muhammed O. Afolabi[1], Dennis Adu-Gyasi[2], Lucy Paintain[1], Theresa Tawiah[2], Mathilda Tivura[2], Samuel Adusi[2], Anthony I. Siibu[2], Elvis Wilson[2], Richmond Kessie[2], Elisha Adeniji[2], Eliezer O. Odei-Lartey[2], Stephaney Gyaase[2], Richard Tetteh[2], Paul Snell[1], Brian Greenwood[1], Daniel Chandramohan[1], Kwaku-Poku Asante[2]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]Kintampo Health Research Centre, Kintampo, Ghana

The overlapping burden of malaria and helminths in LMICs necessitates integrated strategies to advance the global elimination goals. We evaluated the safety, effectiveness, and cost-effectiveness of integrating SMC with anthelminthic codelivery among school-aged children in Ghana. We conducted a cluster-randomised controlled trial with an embedded economic evaluation in central Ghana (July-December 2024). Children aged 5-10 years were randomised at the school level (1:1). On day 0, trained study staff administered a single dose of albendazole and praziquantel to the intervention arm; and the control arm received Vitamin A and Zinc supplements. Following days, the same study staff administered standard SMC (sulphadoxine-pyrimethamine on Day 1; amodiaquine on Days 1-3) to all participants. Finger-prick blood, urine and stool samples were collected pre-intervention and one month post-final SMC cycle for Hb quantification, microscopy and PCR detection of *P.falciparum* and helminth infections. Adverse events (AEs) were monitored via active (6 consecutive days) and passive surveillance. A provider-perspective cost-analysis compared integrated versus standalone delivery. Transient, mild-to-moderate vomiting occurred more frequently in the intervention cluster after albendazole-praziquantel coadministration (0.9%, 6/700) than in the control cluster (0.3%, 2/721). Among 1421 enrolled children (intervention n=700; control n=721), anaemia and malaria prevalence improved in both arms ($p<0.001$). Helminth prevalence reduced significantly only in the intervention arm (between-group difference $p<0.001$). Integrating anthelmintic MDA with SMC was safe, tolerated, and effective in reducing malaria-helminth co-infection and associated morbidity. Expanding SMC to older children incurred additional costs but yielded additional malaria reduction; integrated delivery offered potential cost savings. These findings provide a robust evidence base for policymakers to adopt integrated delivery strategies to maximise public health impact in vulnerable paediatric populations in malaria-endemic, low-resource settings.

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DETERMINANTS OF MALARIA-HELMINTH CO-INFECTIONS AND EFFECTS OF CO-ADMINISTERING SEASONAL MALARIA CHEMOPREVENTION (SMC) DRUGS AND ANTHELMINTICS AMONG SCHOOL-AGED CHILDREN IN PRU EAST DISTRICT, GHANA.

Samuel Adusi Poku[1], Dennis Adu-Gyasi[2], Mathilda Tivura[2], Anthony Ibrahim Siibu[2], Elvis Wilson[2], Richmond Kessie[2], Elisha Adekunle Adeniji[2], Eliezer Ofori Odei-Lartey[2], Stephaney Gyaase[2], Tetteh Richard[2], Lucy Paintain[3], Tawiah Theresa[2], Paul Snell[3], Brian Greenwood[3], Daniel Chandramohan[3], Alexander Manu[1], Muhammed Olanrewaju Afolabi[3], Kwaku Poku Asante[2]

[1]Department of Epidemiology and Disease Control, University of Ghana, School of Public Health, Accra, Ghana, Accra, Ghana, [2]Kintampo Health Research Centre and Development Division, Kintampo North, B/A, Ghana, [3]London School of Hygiene & Tropical Medicine, London, United Kingdom

Ghana is co-endemic for malaria and helminth infections, placing school-aged children at heightened risk of co-infection and associated consequences. This study characterized socio-demographic and WASH determinants of malaria-helminth co-infections and evaluated the effect of co-administering Seasonal Malaria Chemoprevention (SMC) drugs with anthelmintics on co-infection burden in central Ghana. Nested within a cluster-randomised controlled trial (cRCT) (Clinicaltrials.gov NCT06182176), 1,421 school-aged children (5-10 years) from 20 communities were randomised 1:1 to a control arm (Vitamin A/Zinc + SMC [SP-AQ]) or intervention arm (ALB-PZQ + SMC [SP-AQ]). Baseline and endline blood, stool and urine samples were tested at 5-month intervals using malaria microscopy, duplicated Kato-Katz, urine sedimentation, and POC-CCA. Questionnaire on co-infections was administered. Co-infection determinants were assessed using logistic regression. At baseline, malaria prevalence was 170/1421(12%), any helminth infection 268/1421(19%), and malaria-helminth co-infection 31/1421 (2.2%). Malaria-helminths endline and baseline co-infection difference was 2% (95% CI: 0.014-0.029, p value=0.001). Age 8-10 years was independently associated with co-infection (AOR 2.16; 95% CI: 1.01-4.65). Private outdoor toilet use, increased malaria odds (AOR 2.59; 95% CI: 1.15-5.82), while recent antimalarial use, was protective (AOR 0.57; 95%: 0.36-0.91). Never washing hands before meals was strongly associated with helminth infection (AOR 36.6; 95% CI: 5.29-253.28). Open-field defaecation was associated with STH (AOR 2.52; 95% CI: 1.07-5.97) and stream/river use (AOR 1.61; 95% CI: 1.03-2.51) and weekly river contact (AOR 1.65; 95%CI: 1.01-2.68) with schistosomiasis. The strong association between WASH deficiencies and infection risk underscores the need for school-based sanitation and hand hygiene interventions as essential complements to chemoprevention to accelerate progress toward malaria and helminth elimination in co-endemic settings by the year, 2030.

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A MULTI-SECTORAL SCHOOL-BASED ASSESSMENT OF STH, SCHISTOSOMIASIS, WASH, AND HPV VACCINATION IN OGUN STATE, NIGERIA

Temitope Michael Ogunbi

Evidence Action Development Initiative Nigeria, Abuja, Nigeria

In resource-constrained settings, integrating school-based surveys may improve cost efficiency while generating actionable data. In Ogun State, Nigeria, Evidence Action integrated a post-treatment impact assessment for soil-transmitted helminth (STH) and schistosomiasis (SCH) with assessments of Water, Sanitation, and Hygiene (WASH) and Human Papillomavirus (HPV) vaccination. A cross-sectional, cluster survey of 2,154 children (5-14 years) across 41 schools in 20 Local Government Areas (LGAs) was conducted. STH and SCH prevalence were estimated using WHO-recommended model-based geostatistics (MBG); WASH data were collected via structured questionnaires aligned with the WHO WASH toolkit for NTD programs; and an HPV vaccination module was administered to 940 eligible girls aged 9-14 years. State-level analysis indicated a 0.996 probability that any STH prevalence was 10-20% and a 0.998 probability that any SCH prevalence was 1-10%, with only light-to-moderate intensity infections detected. For STH, the mean prevalence at the state level decreased from 34.8% to 12.8% - a 63.3% rate reduction. For SCH, S. haematobium prevalence declined statewide from 25% to 5.2%, a 79.1% reduction. Open defecation (18%) and open urination (34%) remained common; HPV vaccination coverage was 50.3% (CI: 47.0%-53.7%), varying widely across LGAs (0%-90.6%); schools were the primary vaccination site (82%) while the main barriers among unvaccinated girls were not being offered the vaccine (51%) or not knowing where to access it (28%). Compared with separate surveys, the integrated design was estimated to reduce implementation costs by 67%. Integrated school-based assessments can generate policy-relevant parasitological, behavioural, and immunization data at lower cost, and may provide a practical model for coordinated surveillance and program improvement in endemic settings.

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ASSESSING THE IMPACT OF FOUR ROUNDS OF COMMUNITYBASED DEWORMING ON SCHISTOSOMIASIS AND SOILTRANSMITTED HELMINTHIASIS IN WESTERN KENYA: RESULTS FROM THE 2026 ENDLINE EVALUATION

Dollycate Wanja Njagi[1], Chitiavi Juma[1], Maurice Reuben Odiere[2], Faith Kathambi Mutegi[3], Hesbon Ochieng Aoko[4], Stella Kepha[5]

[1]AMREF Health Africa, Kenya, Nairobi, Kenya, [2]Kenya Medical Research Inst, Kisumu, Kenya, [3]Nairobi, Kenya, [4]Kisumu, Kenya, [5]Kenya Medical Research Institute, Nairobi, Kenya

Soil-transmitted helminths (STH) and schistosomiasis (SCH) are public health priorities in western Kenya. An ongoing program delivered four rounds of community-wide mass drug administration (MDA) complemented by WASH and behavior change communication across four endemic counties (Bungoma, Kakamega, Trans Nzoia, Vihiga). An endline evaluation in 2026 assessed cumulative impact on SCH and STH prevalence and intensity among school-age children (SAC). A cross-sectional survey in 72 sentinel schools examined 4,365 SAC (aged 8-14). Single stool samples were processed using duplicate Kato-Katz slides to quantify Schistosoma mansoni, STH (Ascaris lumbricoides, Trichuris trichiura, hookworm), and Taenia spp. Prevalence, mean intensity (epg), and relative reductions were calculated with 95% CIs. Overall S. mansoni prevalence reduced by 88% from 10.7% (2021 baseline) to 1.3% at endline ($Z = 11.5$, $P < 0.001$), with no heavy-intensity infections. This success was widespread: 26 of 32 sub-counties achieved ≥90% reduction, and 53 of 72 schools (74%) recorded zero infections. Overall STH prevalence dropped 16.0% (8.1% to 6.8%; $P = 0.0011$), but this masks species-specific heterogeneity. Hookworm prevalence fell 71.4% ($P < 0.001$) in several sub-counties. Conversely, A. lumbricoides prevalence declined only 12.9% (7.0% to 6.1%; $P = 0.0267$), while mean intensity rose 64%. School-level analysis identified 26 schools (36%) where A. lumbricoides prevalence increased or stayed unchanged, including several in Kakamega and Vihiga. In Vihiga, prevalence rose from 9.4% to 12.3%, and moderate-to-heavy STH infections increased from 3.4% to 3.9%. Taenia spp. were detected at low levels (0.18%) in three counties. Four MDA rounds achieved remarkable SCH reduction, with 74% of schools showing no SCH—signaling progress toward transmission interruption. However, A. lumbricoides persistence with rising intensity in over a third of schools demands adaptive strategies: alternative treatments, targeted WASH in hotspots, integration into routine services, and sustained surveillance.

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EVALUATION OF IMPACT OF BILHARZIA STORYTELLING ON COMMUNITY AWARENESS OF SCHISTOSOMIASIS AMONG PRIMARY SCHOOL-AGED CHILDREN IN BUGESERA DISTRICT, RWANDA, 2024

Bertrand Byishimo[1], Johannes Waltz[2], Ladislas Nshimiyimana[3], Namita Ullegadi[4], Eugene Ruberanziza[5], Jean Bosco Mbonigaba[3], Aimable Mbituyumuremyi[3], Celine Joyeuse Mbabazi[6], Yvonne Ninyembabazi[6], Emile Twagirumukiza[6], Eric Niyongira[7], Didier Nsanzimfura[6]

[1]Emory University, Rollins School of Public Health, Atlanta, GA, United States, [2]Merck, LONDON, United Kingdom, [3]Rwanda Biomedical Center, Kigali, Rwanda, [4]Merck healthcare, Frankfurt, Germany, [5]End Fund, New York, NY, United States, [6]Bilharzia Storytelling Lab, Kigali, Rwanda, [7]Rwanda NGOs Forum, Kigali, Rwanda

Schistosomiasis remains a major public health concern in low- and middle-income countries, particularly among school-aged children in endemic regions such as Rwanda, where Schistosoma Mansoni infection persists despite high school-based mass drug administration coverage. Continued reinfections highlight the need for complementary strategies to address low awareness among children and teachers. To evaluate an innovative storytelling-based education program, a pre- and post-intervention cross-sectional study was conducted among students aged 7–15 in Bugesera District, an area highly susceptible to schistosomiasis. Data were collected from 341 students at baseline and 303 at end line using semi-structured questionnaires assessing knowledge, attitudes, and practices. Awareness of schistosomiasis increased from 14.7% to 98.3%, and knowledge of transmission through water contact rose from 3.8% to 92.1%. Behavioral improvements included a reduction in swimming in open water (35.8% to 7.3%), an increase in toilet use (76.5% to 89.8%), and a decrease in reliance on unsafe drinking water sources (33.7% to 15.5%). Risk perception improved while fear declined, indicating deeper understanding. These findings demonstrate that storytelling-based interventions substantially enhance schistosomiasis-related knowledge and behaviors and offer strong potential for scalable implementation in endemic settings.

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PREVALENCE AND GEOGRAPHIC DISTRIBUTION OF MYCETOMA IN ETHIOPIA: FINDINGS FROM A COMMUNITY-BASED CROSS-SECTIONAL SURVEY

Wendemagegn Enbiale[1], **Dereje Bedane Hunde**[1], Alemayehu Bekele[2], Caesar Olima[3], Stephen Barasa[3], Samuel Teshome Tesema[3], Thaddaeus Egondi3[3], Borna Achieng Nyaoke[3]

[1]Bahir Dar University, Bahir Dar, Ethiopia, [2]Arba Minch University, Arba Minch, Ethiopia, [3]Drugs for Neglected Diseases initiative, Nairobi, Kenya

Mycetoma is a chronic neglected tropical disease causing progressive morbidity and disability, mainly affecting impoverished populations in tropical regions. Ethiopia lies within the "Mycetoma Belt," but reliable population-level prevalence estimates are scarce, limiting effective control strategies. This study estimated prevalence and described epidemiological distribution of mycetoma in Ethiopia and assessed a community-based screening approach with tele-dermatology. A community-based cross-sectional survey was conducted in four regions (Afar, Amhara, Southern Ethiopia, and Tigray). Trained health extension workers conducted house-to-house screening in eight districts across 40 kebeles (the smallest administrative units) using a standardized algorithm. Suspected cases underwent centralized tele-dermatology review; selected cases were referred for confirmation by dermatologists and radiologists using ultrasonography. Of 102,119 individuals screened (49% male, 51% female; mean age 25 years, SD 18.7), 120 suspected cases were identified at the community level. After tele-dermatology review, 68 were selected for further evaluation; 63 consented to referral and underwent ultrasound evaluation, while 5 declined. 55 were confirmed as having mycetoma based on ultrasonographic findings and clinical assessment. Estimated prevalence was 54 per 100,000 (95% CI: 41-70). Afar region had the highest prevalence (157 per 100,000) compared with Southern Ethiopia (22 per 100,000), Amhara (8 per 100,000), and Tigray (8 per 100,000). The screening algorithm's positive predictive value was 49.5% at the health extension worker level, increasing to 87.3% after tele-dermatology review. Confirmed cases were predominantly male (89.1%) and most commonly affected the foot, with 94.5% reporting functional limitations and disability. These findings provide the first population-based mycetoma burden estimate in Ethiopia and show that integrating tele-dermatology with the Health Extension Worker program can substantially improve detection of skin NTDs in resource-limited settings.

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BEYOND THE THORN: KNOWLEDGE GAPS, PERSISTENT MYTHS AND HEALTHCARE SYSTEM BARRIERS AFFECTING MYCETOMA CARE IN TURKANA COUNTY, KENYA

Alice Sinkeet[1], Clare Amuyunzu[1], Mary Kigasia Amuyunzu-Nyamongo[2], Milton Bwibo[1], John Ekai[3], Borna Achieng Nyaoke[4], Olga Mashedi[5], Ruth Nyangacha[5], Diana Kinagu[1]

[1]African Institute for Health & Development, Nairobi, Kenya, [2]African Institute for Health and Development, Nairobi, Kenya, [3]Lodwar County Referral Hospital, Turkana, Kenya, [4]Drugs for Neglected Diseases Initiative, Nairobi, Kenya, [5]Kenya Medical Research Institute, Nairobi, Kenya

Mycetoma is a neglected tropical disease characterized by chronic, progressive swelling and deformity that can lead to severe disability if untreated. In Turkana County, mycetoma prevalence is estimated at 1.46 per 100,000 people, linked to environmental exposure, limited access to healthcare, and low community awareness. The arid climate, barefoot walking, and over-reliance on subsistence livelihoods heighten vulnerability. This study aimed to assess the social, behavioural, and economic determinants affecting mycetoma prevention, healthcare-seeking behaviour, and management. A mixed-methods approach integrating quantitative and qualitative data sources. Participants comprised of individuals affected by mycetoma, community members, local leaders, traditional healers, and healthcare workers. Quantitative data were collected using household questionnaires, WHOQoL, and Cost of Illness tools. Qualitative data was obtained through IDIs, KIIs, and FGDs. Ethical approval was obtained from AMREF ESRC. Knowledge of mycetoma was low with only 31.5% of participants demonstrating good knowledge. Local beliefs were prominent as 25.7% attributed the disease to witchcraft, posing a barrier to healthcare-seeking. Quality of life was significantly lower among affected individuals (mean 31.9) compared to unaffected individuals (mean 50.5) ($p = 0.002$), due to physical and functional limitations. Stigma from family and community members was also reported. Key barriers to accessing care included financial constraints, long distances to health facilities, lack of diagnostic capacity, mistrust of healthcare workers, and fear of amputation. Mycetoma remains a highly neglected and debilitating condition in Turkana County. Limited knowledge, inadequate diagnostic capacity, mistrust in healthcare systems, poverty, and geographic barriers delay diagnosis and treatment. Addressing these challenges requires integrated, community-informed strategies to improve awareness, early detection, and access to care.

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ADVANCING SUSTAINABLE ELIMINATION OF NEGLECTED TROPICAL DISEASES THROUGH DOMESTIC RESOURCE MOBILIZATION: LESSONS FROM DEWORMING INNOVATION FUND IN WESTERN KENYA.

Wyckliff Peter Omondi[1], Irene Natalie Chami[2], Chitiavi Josphat Mmasi Juma[3]

[1]Kenya National Public Health Institute-Ministry of Health, Nairobi, Kenya, [2]The END Fund, New York, NY, United States, [3]254, Kenya

Neglected Tropical Diseases (NTDs) continue to be of public health burden in Kenya contributing to morbidity, disability and poverty. While substantial progress has been made towards elimination, the gains have largely been through external funding. Domestic resource mobilization is desirabel to safeguard the gains made. Aligned with Kenya Public Health Finance

Act, a participatory engagement approach was used to include priority NTDs into county planning and budgeting processes. This involved multi-stakeholder sensitization, targeted advocacy and strategic engagement during critical budgeting cycle. The process saw inclusion of NTDs into Annual Development Plans and County Fiscal Strategy Papers, allowing public participation, hearings and approvals with expenditure tracked through routine reporting systems. Over the last four years, gradual increase in domestic allocation to NTD related activities was observed in the health budgets of the 4 counties. The allocated funding majorly supported implementation of preventive measures, procurement of health products and vector control programs. However, domestic contributions remain insufficient, accounting for less than half of total program financing in most endemic areas. Key enablers included strong political commitment, in-depth knowledge and understanding of budgeting processes, integration of NTD indicators into routine health systems and alignment with Universal Health Coverage priorities. Nonetheless, challenges including; weak or inadequate policy to ring fence NTD allocation, fragmented financing streams, and inadequate tracking of NTD specific expenditures persisted. Domestic resource mobilization is critical to sustaining progress toward NTD elimination. Strengthening NTDs budgetary inclusion and allocation, enhancing financial tracking and institutionalizing integrated service delivery are key in reducing reliance on donor funding. The findings provide insights for policymakers and partners seeking to build resilient and self-sustaining NTD programs not only in Kenya but also similar settings.

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EFFECT OF MATERNAL HEALTHCARE CONTINUUM ON NEONATAL MORTALITY IN SUB-SAHARAN AFRICA: A POOLED DHS ANALYSIS

Benedicta Owusu-Arthur[1], Rita Asante Kusi[2], Duah Dwomoh[1], Ernest Kenu[3]

[1]University of Ghana School of Public Health, Accra, Legon, Ghana, [2]Ghana Field Epidemiology and Laboratory Training Programme, School of Public Health, Accra, Legon, Ghana, [3]Univ of Ghana, Accra, Ghana

Neonatal mortality remains a significant public health concern in sub-Saharan Africa (SSA), accounting for approximately 43% of global neonatal deaths. Continuum of maternal healthcare aims to reduce preventable neonatal deaths; however, existing studies focus on individual countries making the cumulative effect on neonatal mortality across SSA limited. This study assessed the effects of the maternal healthcare continuum on neonatal mortality across countries in SSA.A pooled cross-sectional analysis of DHS data from 35 Sub-Saharan African countries (2010 -2025) conducted among women aged 15–49 years with at least one live birth in the five years preceding each survey. Weighted proportions were computed to describe the distribution of neonatal mortality by country and sub-region. Multilevel logistic regression models examined the effect of continuum of maternal healthcare on neonatal mortality. G-computation and population attributable fractions (PAF) estimated the counterfactual population-level impact. A total of 831,167 women were included, of whom 22,857 (2.75%) experienced a neonatal death. The pooled neonatal mortality rate was 27.7 per 1,000 live births (95% CI: 27.2–28.2) ranging from 3.3 per 1,000 in Comoros to 163.3 per 1,000 in Nigeria. Less than one-third of the women, 23.2% (119,203/513,112) had completed the continuum of care. Highest adherence was reported in Southern Africa, 36.6% (3,592/9,816). Full continuum adherence was associated with an 18% reduction in neonatal mortality odds (aOR: 0.82; 95% CI: 0.76–0.88; p<0.001). Universal coverage would reduce the neonatal mortality rate from 20.3 to 16.3 per 1,000 live births, with the greatest benefit for adolescent mothers (6.4 per 21,000), high-parity mothers (5.5 per 1,000), and women in Western Africa (4.5 per 1,000).Full adherence to the maternal healthcare continuum significantly reduces neonatal mortality across Sub-Saharan Africa, yet fewer than one in four women complete all three components. Strengthening integrated maternal and newborn care, especially in Middle Africa, is crucial for accelerating progress toward SDG 3.2 targets.

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CHILD MORTALITY ATTRIBUTED TO *PNEUMOCYSTIS JIROVECII* IN AFRICA AND ASIA: RESULTS FROM THE CHAMPS NETWORK USING MINIMALLY INVASIVE TISSUE SAMPLING

David Torres-Fernandez[1], Zachary Madewell[2], Sana Mahtab[3], Inacio Mandomando[4], Rosauro Varo[5], Natalia Rakislova[6], Laura Garcia-Otero[7], Sara Ajanovic[8], Portia C. Mutevedzi[9], Dianna M. Blau[10], Cynthia G. Whitney[9], Muhammad F. Bashir[11], Glory Bassey[12], Ubong Udoh[12], Ikechukwu U. Ogbuanu[13], Adetola Daramola[14], Hellen Muttai[15], Nega Assefa[16], Lola Madrid[16], Samba O. Sow[17], Karen L. Kotloff[18], Shams El Arifeen[19], Emily S. Gurley[20], Shabir A. Madhi[21], Dangor Ziyaad[22], Quique Bassat[23]

[1]ISGlobal (Barcelona Institute for Global Health), Barcelona, Spain. Centro Investigação em Saúde de Manhiça (CISM), Maputo, Mozambique. Facultat de Medicina i Ciències de la Salut, Universitat de Barcelona (UB), Barcelona, Spain, [2]Hubert Department of Global Health, Rollins School of Public Health, Emory University. Global Health Center, US Centers for Disease Control and Prevention, Atlanta, GA, United States, [3]Global Health Center, US Centers for Disease Control and Prevention, Atlanta, Georgia, USA. Task Force for Global Health., Atlanta, GA, United States, [4]Centro Investigação em Saúde de Manhiça (CISM), Maputo, Mozambique. Instituto Nacional de Saúde (INS), Maputo, Mozambique, [5]Centro Investigação em Saúde de Manhiça (CISM), Maputo, Mozambique. ISGlobal, Barcelona, Spain. CIBER de Epidemiología y Salud Pública, Instituto de Salud Carlos III, Madrid, Spain. Institut de Recerca Sant Joan de Deu, Hospital Sant Joan de Deu, Barcelona, Spain, [6]ISGlobal, Barcelona, Spain. Facultat de Medicina i Ciències de la Salut, Universitat de Barcelona (UB), Barcelona, Spain. CIBER de Epidemiología y Salud Pública, Instituto de Salud Carlos III, Madrid, Spain. Pathology Department, Hospital Clínic, Barcelona, Spain, [7]ISGlobal (Barcelona Institute for Global Health), Barcelona, Spain; Centro Investigação em Saúde de Manhiça (CISM), Maputo, Mozambique, [8]ISGlobal. Centro Investigação em Saúde de Manhiça (CISM). Universitat de Barcelona (UB), Barcelona, Spain. Pediatric Infectious Diseases and Immunodeficiencies Unit, Vall d'Hebron Barcelona Hospital Campus. Vall d'Hebron Research Institute, Barcelona, Spain, [9]Task Force for Global Health, Atlanta, GA, United States, [10]Global Health Center, US Centers for Disease Control and Prevention, Atlanta, GA, United States, [11]Abubakar Tafawa Balewa University Teaching Hospital and Abubakar Tafawa Balewa University, Bauchi, Nigeria, [12]Department of Paediatrics, University of Calabar, Calabar, Nigeria; Department of Paediatrics, University of Calabar Teaching Hospital, Calabar, Nigeria, [13]Hubert Department of Global Health, Rollins School of Public Health, Emory University, Atlanta, GA, USA. The Africa Research Collaborative (ARC), Freetown, Sierra Leone, [14]The Africa Research Collaborative (ARC), Freetown, Sierra Leone, [15]Kenya Centre for Global Health Research Kisumu, Kenya; Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [16]College of Health and Medical Sciences, Haramaya University, Harar, Ethiopia. Department of Infectious Disease Epidemiology, London School of Hygiene and Tropical Medicine, London, United Kingdom, [17]Centre pour le Développement des Vaccins, Ministère de la Santé, Bamako, Mali, [18]Department of Pediatrics and Department of Medicine, Center for Vaccine Development and Global Health, University of Maryland School of Medicine, Baltimore, MD, United States, [19]International Center for Diarrhoeal Disease Research, Dhaka, Bangladesh, [20]International Center for Diarrhoeal Disease Research, Dhaka, Bangladesh. Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [21]South African Medical Research Council Vaccines and Infectious Diseases Analytics Research Unit, University of the Witwatersrand, Johannesburg, South Africa. Wits Infectious Diseases and Oncology Research Institute, Faculty of Health Sciences, University, Johannesburg, South Africa, [22]South African Medical Research Council Vaccines and Infectious Diseases Analytics Research Unit, University of the Witwatersrand, Johannesburg, South Africa, [23]ISGlobal. Centro Investigação em Saúde de Manhiça (CISM), Maputo, Mozambique. Universitat de Barcelona (UB). CIBER de Epidemiología y Salud Pública, Instituto de Salud Carlos III. ICREA. Hospital Sant Joan de Deu. Hospital Clinic de Barcelona, Barcelona, Spain

Pneumocystis jirovecii is an important cause of childhood pneumonia deaths in low- and middle-income countries (LMICs) endemic for HIV. This study aimed to characterize *P. jirovecii*-associated mortality among children under 5 years of age using clinical data, minimally invasive tissue sampling (MITS), molecular pathogen detection, histopathology, and standardized

cause-of-death attribution in six sub-Saharan African countries and one Asian country from 2016-2023, within the CHAMPS network. Among 1,683 deaths included, *P. jirovecii* was detected in 235 (14.0%) and considered in the causal chain leading to death in 50/235 (21.3%, 3.0% of all deaths). Most children with *P. jirovecii* in the causal chain were aged 1-6 months (35/50, 70.0%) and had coexisting comorbidities like severe undernutrition (26/50, 52.0%), HIV infection (22/50, 44.0%), or low birth weight (16/50, 31.8%). Among children living with HIV with *P. jirovecii* in the causal chain, 3/22 (13.6%) had a record of taking co-trimoxazole prophylaxis. Predominating clinical presentation was lower respiratory tract infection with respiratory distress (41/50, 82.0%). Pathological evidence of *P. jirovecii* in lung tissue (39/50, 78.0%; mainly as interstitial pneumonitis with methenamine silver-positive organisms) supported its causal role compared with other molecular biology detections considered as non-pathological colonization. Coinfections occurred in 90.0% of cases, most commonly with bacteria (74.0%; mainly *Klebsiella pneumoniae* [44.0%] and *Streptococcus pneumoniae* [24.0%]), HIV and cytomegalovirus (40.0% each). Overall, 41/50 (82.0%) of *P. jirovecii*-related deaths were considered preventable, mainly through health education (16/36, 44.4%), HIV prevention (15/36, 41.7%), better clinical management (13/36, 36.1%), and nutritional support (11/36, 30.6%). *P. jirovecii* represents a relevant and preventable cause of severe pneumonia and death in vulnerable children in LMICs. Increasing access to specific diagnostic and therapeutic tools and strengthening healthcare delivery, particularly for HIV and undernutrition is essential to reduce mortality.

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PREVALENCE AND FACTORS ASSOCIATED WITH LOW BIRTH WEIGHT AMONG STILLBIRTHS AND EARLY INFANT DEATHS IN SUB-SAHARAN AFRICA AND SOUTHEAST ASIA, DECEMBER 2016 TO SEPTEMBER 2023: ANALYSIS FROM THE CHILD HEALTH AND MORTALITY PREVENTION SURVEILLANCE NETWORK

Tadesse Gure Eticha[1], Kyu Han Lee[2], Dianna Blau[1], Nega Assefa[1], Florence Murila[3], Emily S. Gurley[4], Kitiezo A. Igunza[5], Hellen Muttai[5], Yasir Yunis[1], Sarah Downs[6], Yasmin Adams[7], Firdose Nakwa[8], Anthony J. Scott[9], Ziyaad Ziyaad Dangor[8], Cynthia G Whitney[10], Portia C. Mutevedzi[10], Ogbuanu Ikechukwu[11], Shabir Madhi[8], Lola Madrid[12]

[1]Haramaya University, School of Medicine, Ethiopia, Harar, Ethiopia, [2]The Task Force for Global Health, Decatur, Georgia, USA, Decatur, Georgia, GA, United States, [3]University of Nairobi, Nairobi, Kenya, [4]Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [5]Liverpool School of Tropical Medicine, Kenya Medical Research Institute (KEMRI), Kisumu, Kenya, [6]MRC Vaccines and Infectious Diseases Analytics Research Unit (VIDA), Chris Hani Baragwanath Academic Hospital, Ethiopia, Soweto, South Africa, [7]7MRC Vaccines and Infectious Diseases Analytics Research Unit (VIDA), Chris Hani Baragwanath Academic Hospital, Soweto, South Africa, [8]MRC Vaccines and Infectious Diseases Analytics Research Unit (VIDA), Chris Hani Baragwanath Academic Hospital, Soweto, South Africa, [9]College of Health and Medical Sciences, Haramaya University, Harar, Ethiopia, Ethiopia, [10]The Task Force for Global Health, Decatur, GA, United States, [11]The Africa Research Collaborative (The ARC), on behalf of the Child Health and Mortality Prevention Surveillance program, Sierra Leone, Sierra Leone, [12]London School of Hygiene & Tropical Medicine, London, United Kingdom

Low birth weight (LBW) is a risk factor for morbidity and mortality in newborns and infants and is linked to chronic medical disorders. This study aimed to determine the prevalence of LBW and its associated factors among stillbirths and early infant deaths enrolled in the Child Health and Mortality Prevention Surveillance (CHAMPS) Network. Using CHAMPS Network data from seven countries (2016-2023), this study assessed LBW prevalence and associated factors among stillbirths and early infant deaths (up to six months). We calculated the prevalence of LBW and described the frequency and characteristics of all stillbirths and early infant deaths. Bivariate and multivariable analyses were conducted to identify child and maternal factors associated with LBW. Among 4,729 stillbirths and early infant deaths analyzed, 61% had LBW, of which 506 (18%) were <1000 grams, 884 (31%) were 1000-1499 grams, and 1402 (49%) were between 1500 and 2499 grams. Compared to deaths without LBW, deaths with LBW were more common in Bangladesh (AOR=4.45, 95% CI= 3.40-5.83), South Africa (AOR=4.506, 95% CI=3.47-5.83), and Ethiopia (AOR=2.24, 95% CI=1.75-2.83) than in other sites, among those born in the community (AOR=1.55, 95%CI=1.15-2.10) rather than in a health facility, and among female babies compared to males (AOR=1.23, 95% CI=1.08-1.40). Other factors statistically associated with LBW among study participants were fetal growth restriction (AOR=21.80, 95% CI=6.81-69.79), multiple gestation (AOR=6.71, 95% CI=5.01-8.99), hypertensive disorders of pregnancy (AOR=2.19, 95% CI=1.83-2.62), placental abruption (AOR=1.96, 95% CI=1.58-2.43), and maternal HIV infection (AOR=1.49, 95% CI=1.19-1.86). CHAMPS data indicate that LBW is a significant characteristic of stillbirths and early infant deaths. Targeted investments in preventing hypertensive disorders of pregnancy, enhancing fetal growth, and improving maternal nutrition are essential to reducing the prevalence of LBW and its associated mortality.

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COMPARATIVE EFFECTIVENESS OF DIFFERENT DOSING SCHEDULES OF PNEUMOCOCCAL CONJUGATE VACCINES AMONG CHILDREN IN BRAZIL: A TARGET TRIAL EMULATION STUDY USING NATIONAL REGISTRIES

Katherine Min Jia[1], Caroline Gava[2], Ana Catarina de Melo Araujo[2], João Carlos Lemos Sousa[2], Fan Bu[3], Shae Gantt[4], Ina Sze-Ting Lee[1], Cristiana Toscano[5], Kayoko Shioda[1]

[1]Department of Global Health, Boston University School of Public Health, Boston, MA, United States, [2]National Immunization Program/Secretariat of Health and Environment Surveillance, Brazilian Ministry of Health, Brasília, Brazil, [3]Department of Biostatistics, University of Michigan, Ann Arbor, MI, United States, [4]Department of Epidemiology, University of Florida, Gainesville, FL, United States, [5]Department of Public Health, University of Goias, Goiânia, Brazil

Pneumococcal conjugate vaccine (PCV) routine immunization is an essential measure to prevent pneumococcal diseases in children. The high cost of these vaccines prompts interest in optimizing dosing schedules to improve cost-effectiveness. However, up-to-date real-world evidence is needed to inform policy. Brazil currently uses a 3-dose schedule recommended by the WHO (2+1: two primary doses + a booster). Considering recent evidence that a reduced 1+1 schedule (one primary dose + a booster) has achieved equivalent disease control in selected settings, we aimed to evaluate the comparative effectiveness of 2+1 vs 1+1 in a large country with a well-established national immunization program, with 52 years of experience and high vaccination coverage. We conducted a nationwide target trial emulation (TTE) among children born in Brazil, 2021-2024. Nationwide live birth, vaccination, hospitalization, and notifiable disease registries were individually linked by the Ministry of Health, covering all municipalities. We applied clone-censor-weight analysis to adjust for immortal time bias and confounding, and compared cumulative incidence by age two. By age two, the cumulative incidence of all-cause pneumonia hospitalization had all children followed the 1+1 schedule was 1.09 times that of had they followed 2+1, but this difference was not statistically significant (relative risk [RR]: 1.09; 95% CI: 0.96, 1.25). For hospitalization due to bacterial meningitis, the RR was 1.21, but this difference was not statistically significant (95% CI: 0.95, 1.61). The cumulative incidence of pneumococcal meningitis was similar between schedules (RR: 0.98; 95% CI: 0.45, 1.88). Further analyses will include death outcomes and use negative control exposures and outcomes to assess and control residual confounding. In this nationwide TTE, a 1+1 PCV schedule did not differ significantly from 2+1 for preventing all-cause pneumonia or bacterial or pneumococcal meningitis hospitalization by age two. TTE is a powerful and practical approach to provide real-world, generalizable evidence on comparative effectiveness of dosing schedules to inform policy.

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RAPID PAPER-BASED RECOMBINASE POLYMERASE AMPLIFICATION FOR SICKLE CELL DISEASE DIAGNOSIS IN LOW-RESOURCE SETTINGS

Mehnaz Urbee Jahangir[1], Alex Kortum[1], Alexis Wilkinson[1], Zoha Wazir[1], Yajur Maker[1], Emilie Novak[1], Venee Tubman[2], Gladstone Airewele[2], Rebecca Richards-Kortum[1]

[1]Rice University, Houston, TX, United States, [2]Texas Children's Cancer and Hematology Centers, Houston, TX, United States

Accurate point-of-care genetic diagnostics are needed to reduce morbidity and mortality from sickle cell disease (SCD) in low-resource settings where laboratory infrastructure is limited. Current diagnostic methods, such as electrophoresis, are expensive and require specialized equipment. Emerging point-of-care tests remain limited in detecting disease in transfused patients and newborns with high fetal hemoglobin. This work reports a paper-based recombinase polymerase amplification (RPA) platform for real time nucleic acid detection of SCD. Paper microfluidic platforms enable cold chain free reagent storage, simplified workflows with minimal fluid handling, and scalable manufacturing for population screening, making them well suited for deployment in low resource settings. RPA reagents have been lyophilized on GF/DVA paper membranes to enable direct amplification from clinical samples. The workflow involves adding lysed buccal swab samples to the membrane, incubating at 39°C for 20 minutes using a compact mechanical heater system, and capturing fluorescence in real time with an in-house imaging system equipped with a monochrome camera. Two allele specific assays targeting βA and βS allele variants were optimized to allow isothermal amplification and real time fluorescence readout on paper. Signal intensity was quantified using custom MATLAB analysis to calculate the amplification driven increase in fluorescence intensity. Successful amplification was confirmed by the formation of fluorescent nucleation sites, demonstrating reliable nucleic acid detection on paper membranes. The platform achieved a limit of detection of 100 copies per reaction and showed robust performance with clinical buccal swab samples. This study establishes a first of its kind rapid, paper-based point-of-care nucleic acid amplification test for SCD and highlights the feasibility of allele specific genetic diagnosis on low cost disposable materials. Future work will include field validation in high prevalence regions such as Malawi. This approach offers a scalable and accessible diagnostic strategy for decentralized SCD screening.

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MULTIDIMENSIONAL HOUSEHOLD POVERTY IS ASSOCIATED WITH MORTALITY AMONG UGANDAN CHILDREN HOSPITALIZED WITH PNEUMONIA: A MIXED-METHODS STUDY

Kimmy Kwok[1], **Joy Yang**[1], Raj Saini[2], Robert Opika Opoka[3], Andrea Conroy[4], Olaro Charles[5], Jackson Amone[5], Juliet Nabwire[6], John Kanyonyozi[7], Sophie Namasopo[8], Michael Hawkes[1]

[1]University of British Columbia, Vancouver, BC, Canada, [2]Simon Fraser University, Vancouver, BC, Canada, [3]Mulago Hospital and Makerere University, Kampala, Uganda, [4]Indiana University, Indianapolis, IN, United States, [5]Ministry of Health, Kampala, Uganda, [6]Global Health Uganda, Kampla, Uganda, [7]Association for Health Innovation in Africa, Kambuga, Uganda, [8]Kabale Regional Referral Hospital, Kabele, Uganda

Background Pneumonia remains the leading infectious cause of death among children under five globally, with the highest burden in low-income countries. We examined the association between household poverty, measured using the Multidimensional Poverty Index (MPI), and in-hospital mortality among Ugandan children with hypoxemic pneumonia. *Methods* We conducted a mixed-methods study at resource-constrained hospitals in Uganda. Quantitatively, we used a standardized questionnaire to calculate household MPI. Qualitatively, focus group discussions (FGDs) with caregivers and nurses of childhood pneumonia survivors explored the social and behavioral dimensions of pediatric pneumonia care. *Results* We included 1,708 children hospitalized with hypoxemic pneumonia (median age 13 months, 43% female, case-fatality rate 2.8%). The median MPI was 0.33 (IQR 0.22-0.50) and 798 households (47%) were classified as MPI-poor. Out-of-pocket payments for the hospitalization constituted a catastrophic health expenditure in 411 cases (25%). A total of 794 caregivers (47%) reported borrowing money from family or friends and 696 (41%) reported selling belongings for the hospital visit. Multidimensional poverty was associated with a 2.1-fold higher risk (95% CI 1.2-3.9, p=0.012) of death. Structural equation modeling suggested that both direct effects and indirect pathways through undernutrition (18% of total effect) and delayed presentation (0.95% of total effect) explained the association between MPI and mortality. Focus group discussions identified barriers to prompt and quality care in three themes: (1) care-seeking costs; (2) deferred biomedicine; and (3) caregiver agency. *Conclusion* MPI is associated with mortality in childhood pneumonia in a low-income setting. The effect is partially mediated through undernutrition and delayed treatment.

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EPI VACCINE COVERAGE AND FACTORS ASSOCIATED WITH DELAYED AND MISSED VACCINATION IN RURAL BANGLADESH

Sampa Dash[1], Eva Sultana[1], Farina Naz[1], Md Ali[1], A.S.G Faruque[1], Subhra Chakraborty[2]

[1]International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Dhaka, Bangladesh, [2]Johns Hopkins University, Baltimore, MD, United States

Immunization of children against vaccine-preventable diseases (VPD) is highly effective in reducing under-five mortality and morbidity. However, despite high EPI coverage, 24,000 children die due to pneumonia annually in Bangladesh. A measles outbreak infected 676 children within the first three months of 2026, while both pneumonia and measles are vaccine preventable. Untimely vaccination may not protective during the period of greatest vulnerability. We assessed vaccination coverage and timeliness, and examined factors associated with delayed vaccination and occurrence of VPD among children. This study analyzed data collected from 2,036 children aged 18-60 months in rural Bangladesh. The immunization timeliness was assessed according to the national EPI schedule. Descriptive analyses were used to summarize vaccine receipt and timeliness. To identify factors associated with delayed and missed vaccination, logistic regression was done, while additional models assessed factors linked to pneumonia and measles. Overall EPI coverage is over 96% for all the vaccines except for the second dose of MR (81.2%). However, timely vaccination was poor: only about one-third received BCG, third doses of pentavalent, PCV, OPV, and second IPV on time. Fewer than 1% received all vaccines within the recommended schedule. Lack of knowledge about catch-up vaccination was strongly associated with missed MR vaccination (aOR = 2.18, 95% CI: 2.12-3.57). Lower maternal education (aOR = 1.57, 95% CI: 1.01-2.42) and preterm birth (aOR = 1.43, 95% CI: 1.07-1.90) were associated with delayed vaccination. In the disease models, pneumonia was associated with preterm birth and instrumental delivery, while measles was associated with lack of exclusive breastfeeding, big baby at birth, and young maternal age. High immunization coverage in this population masks major gaps in timeliness. Maternal education, prematurity, and catch-up knowledge were the important determinants of timely vaccination. Programmatic efforts should shift beyond coverage alone and strengthen reminder, follow-up, and catch-up systems to reduce delayed and missed vaccinations.

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ADOLESCENT PREGNANCIES, CHILD HEALTH BEHAVIORS AND OUTCOMES IN SUB-SAHARAN AFRICA

Bolanle Olapeju[1], Michael Bride[2], Milly Nakatabira[3], Zoe Mistrale Hendrickson[4]

[1]Uniformed Services University of the Health Sciences, Bethesda, MD, United States, [2]Johns Hopkins University, Arlington, VA, United States, [3]Johns Hopkins University, Baltimore, MD, United States, [4]University of Pittsburgh School of Public Health, Pittsburgh, PA, United States

With twice the global rate, adolescent pregnancy in sub-Saharan Africa remains a major public health concern, with severe long-term consequences for both mother and child. We investigate the effects of adolescent pregnancy on child health behaviors and outcomes. We assessed whether: i) adolescent mothers (≤19 years) were less likely to adopt healthy behaviors such as skilled birth delivery, immediate breastfeeding, and vaccination, compared to older mothers; and ii) children born to adolescent mothers had a higher risk of adverse health outcomes, including infant mortality and moderate/severe anemia, compared to those born to older mothers. Data were drawn from mothers of singleton live births in the past year (N = 46,551) in recent (2018-2023) Demographic and Health Survey (DHS) data across 19 African countries. Multivariable logistic regressions controlled for sociodemographic factors, healthcare utilization self-efficacy, decision-making autonomy, country, and survey year. Adolescent mothers were more likely to have a secondary education (AOR: 1.38; 95% CI: 1.21, 1.58) but were significantly less likely to be married (AOR: 0.29; 95% CI: 0.24, 0.36), have decision-making autonomy (AOR: 0.81; 95% CI: 0.71, 0.98), or live in the wealthiest households (AOR: 0.25; 95% CI: 0.20, 0.31). Furthermore, adolescent mothers were less likely to utilize skilled birth deliveries (AOR: 0.77; 95% CI: 0.67, 0.87) or vaccinate their children (AOR: 0.78; 95% CI: 0.67, 0.90). Their children were more likely to experience recent diarrhea (AOR: 1.30; 95% CI: 1.14, 1.50) and infant mortality (AOR: 1.88; 95% CI: 1.38, 2.57). Early motherhood may contribute to suboptimal child health due to biological immaturity, limited decision-making autonomy, and economic instability, despite higher educational attainment. These findings inform the need for targeted policies to improve autonomy and economic opportunities for adolescent mothers. School-based, youth-friendly interventions should seek to improve health literacy, autonomy, and the timing and spacing of pregnancies to optimize child health outcomes.

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CRAMPS OUTPACE BLEEDING 5-TO-1 AS THE PRIMARY MENSTRUAL DISRUPTOR: A COMMUNITY SURVEY OF 907 WOMEN IN KENYA

Nadia N. Tsado, Ann Messer, Gabriela Lesiv, Addison Scales, Shayna Kushner

One Good Turn Global Health, West Lake Hills, TX, United States

Menstrual hygiene management (MHM) programs in low- and middle-income countries focus on bleeding-related needs such as sanitary product access, while menstrual pain remains largely unaddressed in school health programming and MHM efforts at large. We conducted a cross-sectional survey of 907 women aged 18+ in Kenya (2024-2025) to quantify the functional burden of menstrual cramps and evaluate whether cramps are perceived as more disruptive than other symptoms. Cramps were the most commonly reported symptom (85.9%) and were identified as the most disruptive to daily life by 80.5% of respondents, five times the rate of bleeding (15.7%). This pattern was consistent across personal disruption (80.5%) and absenteeism (81.3%). Mean pain severity was 4.87/10 (SD=2.89). For each one-point pain increase was associated with 18% more days of school/work missed (IRR=1.18, 95% CI 1.13-1.23, p<.001). Cramp frequency was also significant: those who sometimes experienced cramps had 31% fewer missed days than those who always did (IRR=0.69, p=.002). Pain severity (aOR=1.39, p<.001) and "always" experiencing cramps (aOR=2.41, p=.003) predicted seeking emergency care for cramps, reported by 16.9% of participants. Nearly half (47.9%) used no medications for cramp pain; among those who used medication, 23.1% reported them ineffective. Over 85% stated effective cramp management would help them stay at school and work. These findings demonstrate that menstrual cramps are a substantial, under-addressed functional burden among Kenyan women, with pain severity driving work and school absenteeism and emergency care assistance. The dominance of cramps over bleeding challenges the current MHM emphasis on bleeding management alone and supports integrating pain management, including medication access, into menstrual health programming.

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SPOROZOITE RECOGNITION BY POTENT ANTIBODIES TARGETING THE CRYPTIC *PLASMODIUM FALCIPARUM* PGLU-CSP EPITOPE

Re'em Moskovitz[1], Nathan Beutler[1], Irene Khalek[1], Eric Ji Da Wang[1], Cherrelle Dacon[2], Yen-Chung Lai[1], Monika Jain[1], Justin Ndihokubwayo[3], Adriano Franco Casin[2], Samantha Petros[2], Mirna Pena[2], Holly McAleese[2], Sarit Radak[1], Jordan Woehl[1], Jessica Coppola[1], Xiaohe Lin[1], Yoojin Kim[1], Gonzalo Gonzalez-Paez[1], Elizabeth A. Winzeler[3], Dennis Burton[1], Joshua Tan[2], Joseph G. Jardine[1], Thomas Rogers[1], Ian A. Wilson[1]

[1]The Scripps Research Institute, La Jolla, CA, United States, [2]National Institutes of Health, Rockville, MD, United States, [3]University of California, San Diego, La Jolla, CA, United States

The cryptic pGlu-CSP epitope on the *Plasmodium falciparum* circumsporozoite protein (*Pf*CSP) is not targeted by any next-generation vaccines or therapeutic monoclonal antibodies (mAbs). Recently, we characterized pGlu-CSP recognition by best-in-class human antibody MAD21-101. Using structural and biophysical methods, we demonstrate the structural basis for unique recognition and stepwise binding of the MAD21-lineage and MAD21-101 to pGlu-CSP. We use yeast display-based saturating scanning mutagenesis and combine enriched point mutations to produce MAD21-101 variants with improved breadth and picomolar affinity to pGlu-CSP *in vitro.* In a murine transgenic sporozoite challenge model, our lead candidates provide protection equivalent to best-in-class *Pf*CSP-targeting mAb L9, highlighting MAD21-101 variants for future development as antimalarial therapeutics. To determine the immunogenicity of pGlu-CSP, mice we were immunized with pGlu-CSP-KLH conjugates, and a panel of pGlu-CSP specific mAbs was isolated using 10X genomics. Multiple antibodies from diverse antibody lineages specifically bound to pGlu-CSP with nanomolar affinity in vitro and protected equivalently to MAD21-101 from in vivo transgenic sporozoite challenge. The crystal structures of pGlu-CSP mAbs reveal a breadth of binding modes to the pGlu-CSP epitope, sequence flexibilities and constraints, similarities between the murine and human antibody repertoire, and delineates a minimal epitope for next-generation vaccine design. This study demonstrates the exciting pGlu-CSP epitope is an immunogenic target for exceptionally potent antibodies and a key target for future antimalarial intervention development.

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PLASMODIUM FALCIPARUM MRNA PRE-ERYTHROCYTIC STAGE ANTIGENS INDUCE KINETICALLY DISTINCT GERMINAL CENTER B CELLS AND CD4 TFH CELL RESPONSES IN MICE

Muhammad Riadul Haque Hossainey[1], Steven Tan[1], Mohamad-Gabriel Alameh[2], Alicia M. Moreau[1], Jonathan P. Renn[3], Alexander Pichugin[1], Kutub Ashraf[1], Alison Roth[1], Drew Weissman[2], Patrick E. Duffy[3], Urszula Krzych[1]

[1]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [2]University of Pennsylvania, Philadelphia, PA, United States, [3]National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD, United States

Malaria causes significant morbidity and mortality globally and remains a major health concern. Despite decades of research, the most advanced *Plasmodium falciparum* (Pf) recombinant protein vaccines, based on circumsporozoite protein (CSP), do not induce durable protection. A

combination vaccine that integrates CSP and liver-stage antigens (LS Ags) may improve the efficacy of CSP-based vaccines. Advancements in mRNA vaccine technology allow for incorporation of multiple immunogens into a single vaccine enabling rapid and efficient immunization strategies. We chose two constructs of LS Ags, Pf TATA-box binding protein (TBP) and Pf serine hydroxy methyl transferase (SHMT), orthologues of protective murine LS Ags, and used them as lipid nanoparticle (LNP)-formulated mRNA immunogens for immunizations. Because germinal center (GC) B cells and CD4 T follicular helper (Tfh) cells play essential roles in the development of strong and effective antibody responses, we evaluated whether these lymphocyte populations are detectable in the draining lymph nodes of C57Bl/6 mice immunized with Pf CSP, Pf SHMT, and Pf TBP as mRNA-LNPs. Kinetics analyses revealed a hierarchical induction of GC B cells. CSP induced the highest percentage of $CD38^{-}GL7^{+}$B cells on day 7, followed by a delayed TBP-induced response, while SHMT-induced responses increased as CSP responses declined. These responses parallel GC formation as determined by Ki-67 immunohistology, serum IgG levels, and *in vitro* IgG inhibition of Pf sporozoite invasion of hepatocytes. In contrast, $CXCR5^{+}PD1^{+}CD4$ Tfh cells were detectable as early as day 4 post-immunization, with the highest frequencies observed in TBP-immunized mice, followed by CSP and SHMT. These findings demonstrate both the temporal and functional uniqueness of immune responses induced by each immunogen and highlight the potential of integrating these antigens into an effective mRNA-LNP vaccine candidate for malaria. Studies are underway to evaluate these antigens for protective immunity in a murine model using *P. berghei* transgenic parasites expressing Pf CSP, TBP and SHMT.

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GENETIC VARIATION OF THE *PLAMODIUM FALCIPARUM* CELTOS GENE ACROSS REGIONS OF VARYING MALARIA TRANSMISSION IN TANZANIA AND ITS IMPLICATIONS FOR VACCINE EFFICACY

Gideoni I. Maulidi[1], Dativa Pereus[2], Angelina J. Kisambale[1], Catherine Bakari[1], Ninja S. Bisaga[3], Hussein J. Semboja[1], Rule Budodo[1], Salehe S. Mandai[1], Baraka W. Mbuba[1], Happyness A. Mwailunga[1], Laurencia S. Saidi[1], Rashid A. Madebe[3], Gervas Chacha[1], Doris Mbata[3], Daniel P. Challe[4], Daniel A. Petro[5], Solomon Mwakasungula[1], Daniel Mbwambo[6], Sijenunu Aaron[6], Abdallah Lusasi[6], Samwel Lazaro[6], Stella Kajange[7], Celine I. Mandara[3], Misago Seth[3], Deus S. Ishengoma[1]

[1]Ifakara Health Institute, Dar es salaam, United Republic of Tanzania, [2]Muhimbili University of Health and Allied Sciences, Dar es salaam, United Republic of Tanzania, [3]National Institute for Medical Research, Dar es salaam, United Republic of Tanzania, [4]National Institute for Medical Research, Tanga, United Republic of Tanzania, [5]University of Dar es salaam, Dar es salaam, United Republic of Tanzania, [6]National Malaria Control Programme, Dodoma, United Republic of Tanzania, [7]Prime Ministers' Office, Regional Administration and Local Government, Dodoma, United Republic of Tanzania

Malaria remains a significant public health concern in Tanzania, where transmission intensity varies across regions, indicating that innovative interventions are urgently needed to progress towards the elimination targets. Understanding genetic diversity of *Plamodium falciparum* antigens is essential for vaccine development. This study examined population-level genetic variation in the *P. falciparum* Cell-Traversal Protein for Ookinetes and Sporozoites (CelTOS) gene across five regions of mainland Tanzania to assess how diversity influences antigen recognition and evaluate its potential as a next-generation malaria vaccine candidate. A total of 589 isolates from five selected regions in Tanzania were retrieved from the MalariaGEN database and analyzed, along with isolates from Kenya (2 sites, n = 690), Ethiopia (2 sites, n = 21), and Uganda (1 site, n = 11). Within Tanzania, 31 segregating sites generated 210 haplotypes (Hd = 0.981, π = 0.00854, K = 4.69), reflecting high haplotype but low nucleotide diversity. Genetic differentiation among the selected Tanzanian regions was low (Fst = 0.0077), indicating that CelTOS is highly conserved, with sequence conservation C = 0.944. Tajima's D was 0.134 (P > 0.10), suggesting neutral evolution. Pairwise Fst between Tanzania and other countries was low for Kenya (Fst ≈ 0.002) and Uganda (Fst ≈ 0.004), but higher for Ethiopia (Fst ≈ 0.137). Population Fst across countries was 0.068, indicating low-moderate differentiation. Chi-square for genetic differentiation among all populations was significant (Chi^2 = 1376.997, P < 0.001), mainly reflecting differences among countries. These findings suggest CelTOS is relatively conserved across Tanzanian *P. falciparum* populations despite high haplotype diversity. However, it remains unclear whether variation occurs in epitope regions; epitope-level analysis is planned to assess its impact on immune recognition and support CelTOS as a vaccine candidate. This study provides evidence-based insights into CelTOS as a malaria vaccine candidate and highlights the importance of regional genetic surveillance in guiding vaccine development.

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DEEP-LEARNING BASED PROTEIN DESIGN OF RETICULOCYTE BINDING PROTEIN HOMOLOG 5 (RH5) IMMUNOGENS

Mark D. Langowski[1], Lloyd DW King[2], Mila Y. Lamb[1], Kirsty McHugh[2], Anna Huhn[2], Francesca Donellan[2], Cassandra Rigby[2], Harriet Parker[2], Doris Quinkert[2], Daniel Pfalmer[1], Adian Valdez[1], Alex Kang[1], Rashmi Ravichandran[1], Asim K. Bera[1], Simon J. Draper[2], Neil P. King[1]

[1]University of Washington, Seattle, WA, United States, [2]University of Oxford, Oxford, United Kingdom

Plasmodium falciparum Reticulocyte binding protein homolog 5 (RH5) is the leading blood-stage malaria vaccine candidate, with the RH5.1 immunogen formulated in Matrix-M recently demonstrating 55% efficacy in children in Phase 2b trials. Though RH5.1 is promising, a few challenges remain in regards to improving its manufacture, stability, and potency. For example, a computationally stabilized variant, RH5.2, has been shown to be more thermostable and expressed at higher yields compared to RH5.1, but suffers from disrupted inhibitory epitopes (Communities III/IV) and can only be SpyCatcher-SpyTag conjugated to virus-like particles at a maximum valency of 25% to avoid precipitation. Thus we wanted to address these problems by improving manufacturability in low cost expression systems such as *E. coli*, restoring antigenicity towards disrupted epitopes, and enabling the display of RH5 immunogens as genetic fusions to nanoparticles or other multimers at 100% valency. To overcome these limitations, we used deep-learning based protein design methods RFdiffusion and ProteinMPNN to generate 1) stabilized RH5 that improve upon RH5.2 while restoring antigenicity which we refer to as RH5.4 and 2) *de novo* RH5 immunogens, which we refer to as RH5 half-diamonds (RH5hd), that scaffold epitopes targeted by inhibitory antibodies in communities I, II, and III to improve the potency of the response by leaving out portions of the RH5 antigen targeted by non-neutralizing antibody communities IV and V. These RH5 immunogens can be expressed in *E. coli* and purified at higher yields compared to RH5.2, restore antigenicity towards critical epitopes, and improve thermostability. X-ray crystallography confirmed high fidelity of designs to RH5 and the precise scaffolding of epitopes. These designs also enabled genetic fusion to 60-mer icosahedral protein nanoparticles at 100% valency. In rat models, these designed immunogens and their fusions to nanoparticles elicited significantly higher growth inhibition assay (GIA) activity compared to RH5.1, offering a more potent, manufacturable, and antigenically intact vaccine platform.

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NEXT-GENERATION PFSPZ VACCINES: DEVELOPMENT OF PAN-CONTINENTAL HYBRIDS FOR ENHANCED ANTIGENIC BREADTH

B. Kim Lee Sim[1], Lucia Pazzagli[2], Bethany Jenkins[3], Asha Patil[1], Yonas Abebe[1], Manuel Llinas[4], Stephen L. Hoffman[5], Joana C. Silva[6], Ashley Vaughan[2]

[1]Sanaria Inc., Rockville, MD, United States, [2]Seattle Children's Research Institute, Seattle, WA, United States, [3]Sanaria, Inc., Rockville, MD, United

States, [4]Pennsylvania State University, University Park, PA, United States, [5]Sanaria Inc, Rockville, MD, United States, [6]Univ of Maryland School of Medicine, Silver Spring, MD, United States

Plasmodium falciparum sporozoite (PfSPZ) vaccines derived from the West African isolate PfNF54 have high manufacturing yields and have achieved 100% efficacy against homologous and heterologous controlled human malaria infection at 10-12 weeks and sustained protection in the field in Africa for 21 months. However, it requires four times as many PfSPZ to achieve 100% protection against heterologous controlled human malaria infection (CHMI) as it does against homologous CHMI. Furthermore, PfNF54 exhibits lower liver infectivity than other strains necessitating higher sporozoite doses thereby increasing cost of goods, and its genetic divergence from non-West African isolates may limit global vaccine efficacy (VE). To address these limitations, we utilized genetic crossing to develop single hybrid parasite strains that combine high manufacturing productivity with enhanced hepatocyte invasion and broader antigenic coverage. We crossed PfNF54 with three East African strains (PfMAL31, PfNF165, PfHL1209), transitioning resulting sporozoites through human liver-chimeric FRG huHep mice to confirm viability. The lead hybrid, AV27, demonstrated balanced parental genomic representation, superior gametocyte and PfSPZ production, and 15-fold enhanced liver infectivity compared to parental strains. To further expand global coverage, AV27 was crossed with the Thai strain NHP4026 to create a "pan-continental" hybrid. Unlike traditional multi-strain cocktails, this genetic recombination strategy yields a single, optimized strain possessing the manufacturing efficiency of PfNF54 and the infectivity and immune breadth of diverse global isolates. These pan-continental hybrids provide a scalable pathway to achieve high, sustained VE across geographically (hence genetically) diverse clinical settings.

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DEVELOPMENT OF MULTI-STAGE MRNA VACCINES FOR *PLASMODIUM VIVAX* MALARIA BASED ON PVCSP AND PVDBP

Alberto Ayllon-Hermida[1], Francisco J. Martinez[1], Racha Al-Radi[1], Katleen Petit[1], Christèle Huon[1], Eric Legrand[1], Rebeca Lampley[2], Hamilton Bennett[2], Jennifer Pham[1], Anthony Bouillon[1], Sabine Thiberge[1], Rogerio Amino[1], Michael White[1], Chetan E. Chitnis[1]

[1]Institut Pasteur, Paris, France, [2]Moderna Tx, Cambridge, MA, United States

Development of vaccines against *P. vivax* is a global health priority, yet it remains considerably less advanced than efforts targeting *P. falciparum*. The unique biology of *P. vivax*, including dormant liver stages and antigenic diversity, complicates the induction of protective immunity. We propose to develop multi-stage mRNA-based vaccines based on two leading antigenic targets: the circumsporozoite protein (PvCSP) to target sporozoites and the Duffy binding protein (PvDBP) to target blood-stage parasites. PvCSP is the major surface protein of the sporozoite and is essential for motility and hepatocyte invasion. We developed mRNA constructs incorporating central repeat regions representative of vk210 and vk247 variants, the N-terminal region, and conserved C-terminal domains to maximize cross-strain coverage. Delivered via mRNA-lipid nanoparticles (mRNA-LNP), PvCSP constructs induced strong humoral responses in mice, with antibodies binding native sporozoites and inhibiting hepatocyte infection leading to high levels of protection of C57BL/6 mice against challenge with transgenic P. berghei expressing vk210 or vk247 PvCSP. To target blood stages, we developed PvDBP-based mRNA vaccines focusing on Region II, which mediates merozoite attachment to the Duffy antigen receptor (DARC) on reticulocytes. Immunization with PvDBPII induced antibodies that inhibit PvDBP-DARC interaction, a validated immune correlate of blood stage protection. Together, these results provide a robust preclinical basis for next-generation multi-stage *P. vivax* vaccines. Combination of PvCSP and PvDBPII mRNA constructs, optimized designs, and dosing strategies are being assessed to identify candidates for clinical development. mRNA-LNP technology enables flexible antigen design, rapid production, and potent immunogenicity, supporting future multivalent malaria vaccines.

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IMMUNOGENICITY AND TRANSMISSION-REDUCING ACTIVITY OF MRNA-LNP VACCINES ENCODING PFS25, PFS230D1M AND PFS230D7D10

Yi Cao[1], Clifford T. H. Hayashi[1], Tucker B. Harju[1], Abhai K. Tripathi[2], Garima Dwivedi[3], Drew Weissman[3], Nirbhay Kumar[1]

[1]George Washington University, School of Public Health, Washington, DC, United States, [2]Johns Hopkins Malaria Research Institute, Baltimore, MD, United States, [3]Department of Medicine, University of Pennsylvani, Philadelphia, PA, United States

Transmission-blocking vaccines targeting sexual-stage development of *Plasmodium* in mosquitoes represent a critical strategy to interrupt malaria transmission. Pfs25 and Pfs230D1M are most advanced vaccine candidates in clinical trials. Pfs230D7D10 is a newly identified candidate in our studies. mRNA-LNP technology provides a rapid, scalable, and effective platform for vaccine development. In this study, we evaluated immunogenicity and transmission-reducing activity (TRA) of mRNA-LNPs encoding Pfs25, Pfs230D1M and Pfs230D7D10. Male and female BALB/c mice received mRNA-LNPs at doses of 10, 5 and 1µg in a 3-immunization regimen at 4-week intervals to determine the optimal dose. We also compared immune responses among 3 mouse strains (BALB/c, C57BL/6, and outbred CFW) at a 5µg dose. Antigen-specific antibody responses (endpoint titers, IgG subclasses, and avidity) were assessed by ELISA. Functional activity of induced antibodies was evaluated in standard membrane feeding assays (SMFAs). All three mRNA-LNPs elicited strong antibody responses with comparable titers across 3 doses, except that 5 µg Pfs230D7D10 elicited a statistically lower titer compared to the 10 and 1µg doses. No significant differences in antibody titers were observed between male and female BALB/c mice, or among 3 mouse strains. Pfs25 mRNA-LNP induced balanced IgG subclasses (assessed as IgG1/IgG2a/c ratio) and similar antibody avidity across doses and mouse strains. Pf230D1M mRNA-LNP elicited IgG1-dominant responses in BALB/c mice. In SMFAs, anti-Pfs25 IgG was highly potent in reducing parasite transmission to mosquitoes, achieving >90% TRAs at IgG concentrations as low as 0.031 mg/mL, regardless of dose, mouse sex, and strain. Anti-Pfs230D1M IgG also exhibited > 83% TRAs at IgG concentrations as low as 0.25 mg/mL. The 1 µg Pfs230D1M showed superior TRA at 0.125 mg/mL compared to 10 and 5 µg groups (97.5% vs 45% vs 88%). In contrast, anti-Pfs230D7D10 IgG demonstrated only moderate TRAs. Collectively, our results demonstrate robust functional immunogenicity of Pfs25 and Pfs230D1M mRNA-LNPs, supporting their further evaluation in nonhuman primates.

6990

A *PLASMODIUM*-DERIVED NANOPARTICLE VACCINE CAN DISPLAY UP TO FOUR DIFFERENT ANTIGENS AND PROVIDES COMPLETE STERILE PROTECTION IN MICE

Thayne H. Dickey, Dashuang Shi, Rui Ma, Richi Gupta, Palak N. Patel, Nichole D. Salinas, Wai Kwan Tang, Alaysies Queen, Myesha Singleton, Nida Delbe, Solomon Conteh, Lynn E. Lambert, Patrick E. Duffy, Niraj H. Tolia

National Institutes of Health, Bethesda, MD, United States

Nanoparticle vaccine platforms, like the hepatitis B surface antigen (HBsAg) used in RTS,S and R21, enhance the immunogenicity of the antigen displayed on the surface. However, these platforms typically derive from organisms unrelated to the vaccine target, and the immune response generated against the platform provides no benefit to the vaccinee. We sought to improve the efficacy of malaria vaccines by developing a nanoparticle platform from exclusively *Plasmodium falciparum* proteins. Pyridoxal 5'-phosphate (PLP) synthase is a 24-copy heterodimeric complex that we engineered to form a stable nanoparticle (P1P2). Cryogenic electron microscopy resolved a 2.95-Å resolution structure of the PLP nanoparticle including amino acid changes engineered to stabilize the nanoparticle. Antigens can be attached to both the N- and C-termini of the P1 and P2 subunits allowing 48 copies of up to 4 distinct antigens to

be displayed. Fusion of an engineered circumsporozoite protein (CSPj5c) and the cell-traversal protein for ookinetes and sporozoites (CelTOS) resulted in stable and immunogenic nanoparticles. Mice were completely protected from parasite challenge after three immunizations. This level of protection appears superior to published RTS,S and R21 data, motivating head-to-head comparisons and pre-clinical development. P1P2 can be recombinantly expressed and purified from *E. coli* as an intact complex making it a cost-effective vaccine candidate. Additionally, the ability to display multiple distinct antigens on P1P2 could enable future development of a single-component multi-stage vaccine or a vaccine targeting multiple species or strains of parasite.

6991

CHANGES IN THE DISCIPLINING OF YOUNG JAMAICAN CHILDREN IN THE HOME FROM 2005 TO 2022

John Duncan McLennan

University of Calgary, Calgary, AB, Canada

The prevalence of child exposure to physical punishments appears to be decreasing in Jamaica while use of nonphysical disciplinary strategies is increasing. However, this is based on crude composite measures that lump disparate disciplinary practices together. Examining changes at the individual disciplinary practice level may provide important details that better inform more nuanced health promotion initiatives to improve child rearing. This study aimed to determine the pattern of change in the prevalence of child exposure to individual disciplinary practices in Jamaica between 2005-2022. Data from Jamaican 2005, 2011 and 2022 nationally representative household surveys (Multiple Indicator Cluster Surveys) were used, specifically, caregiver responses to the previous month's exposure of a randomly selected child (24-59 months of age) to 6 physical punishments and 3 non-physical disciplinary strategies. Samples sizes were 443, 525, and 706 for the 2005, 2011, and 2022 surveys, respectively. "Spank", the most common physical punishment at each time point, decreased across surveys from 66.8% to 62.2% to 54.1%. The steepest decrease (-1.09%/year) was seen for "Hit with object", dropping from 30.4% in 2005 to 11.8% in 2022. The only exceptions for decline in use of physical punishments across time were slight increases in "Hit limb" and "Hit head" between 2011 and 2022. The decreases were steeper between 2005-2011 than 2011-2022 for all physical punishments. Changes for nonphysical strategies were more variable. There was a slight reduction in "Explain wrong behavior" across the three time points (80.0% to 76.5% to 72.2%). In contrast, "Take away privileges" and "Give something else to do" decreased between 2005 and 2011, but then both ended at their highest prevalence in 2022. The variability in change between disciplinary types over time supports the proposition that analysis of disciplinary trends should not exclusively occur at the composite level. A next step is determining what underpins these variable changes (e.g., impact of legislation, changes in caregiver attitudes, impact of educational initiatives).

6992

INTERVENTION-SPECIFIC, TEMPORALLY DISTINCT INFLAMMATORY PROTEOME SIGNATURES OF PRO/SYNBIOTICS IN KENYAN INFANTS: EVIDENCE FROM THE PROSYNK TRIAL

Mary Iwaret Otiti[1], Simon Kariuki[1], Alloys K'Oloo[1], Micah J. June[1], Miriam W. Chomba[1], Kephas Otieno[1], Tyson H. Holmes[2], Feiko ter Kuile[3], Stephen Allen[3], Holden T. Maecker[2]

[1]Kenya Medical Research Institute- Center for Global Health Research (KEMRI-CGHR), Kisumu, Kenya, [2]Stanford University School of Medicine, Institute for Immunity, Transplantation and Infection, Palo Alto, CA, United States, [3]Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Early-life pro/synbiotic interventions may shape the developing immune system, yet their effects on the inflammatory proteome in African infants remain poorly defined. In the PROSYNK trial, three pro/synbiotics markedly reduced chronic systemic inflammation (plasma α_1-acid glycoprotein, peak at 6 months) in Kenyan infants. High-dimensional proteomics on stored plasma using NULISAseq proximity ligation assay yielded 249 proteins quantified in 559 infants with samples at 6 weeks, 3, 6 and 12 months. Cross-sectional quantile regression at the 25th, 50th, and 75th percentiles with multiple testing correction per arm-by-visit stratum was the primary pre-specified analysis. Each arm produced a distinct temporal and distributional immune signature with effects concentrated at the 75th percentile, indicating greatest immunomodulation in infants with the highest baseline inflammation. Labinic Synbiotic demonstrated the broadest effect, with 14 confirmed proteins (12 upregulated) at 6 months, including checkpoint CTLA4 (β=0.238, q=0.0001), mucosal cytokine IL-22 (β=0.857, q=0.024), angiogenic receptor TEK (β=0.140, q<0.001), and costimulatory molecules CD80 (β=0.195, q=0.004) and IL12RB1 (β=0.268, q=0.025), indicating T-cell regulation and mucosal immune engagement. Lab4b Synbiotic produced an earlier response at 6 weeks and 3 months, with 12 confirmed proteins (11 upregulated) including IL-18R1 (β=0.220, q=0.002), IL-2Rα (β=0.175, q=0.007), MMP3 (β=0.266, q=0.022), and IL-7 (β=0.586, q=0.008), implicating innate immune activation and lymphopoietic priming in the neonatal colonisation window. Lab4b Probiotic showed a more targeted effect with 4 upregulated proteins: IL1RL1 (β=0.333, q=0.027) at 6 weeks, CXCL12 (β=0.391, q=0.039) at 3 months, and PDCD1LG2 (β=0.188, q<0.001) and TNFSF4 (β=0.140, q=0.016) at 6 months. Canonical markers CRP, TNF, IL-6, and IL-10 showed no significant treatment effect, consistent with selective immunomodulation rather than broad suppression. These proteome-level findings support the role of pro/synbiotics in early immune programming in low- and middle-income settings.

6993

CAUSES OF DEATH AMONG CHILDREN UNDER FIVE IN KARACHI PAKISTAN: COMMUNITY-BASED FINDINGS FROM 150 MINIMALLY INVASIVE TISSUE SAMPLING (MITS) CASES IN THE CHAMPS NETWORK

Abdul Momin Kazi[1], Raheel Allana[1], Mahder Tracey[2], Zeeshan Ahsan[1], Areeba Sultan[1], Zeeshan Uddin[1], Shabina Ariff[1], Fauzia Aman Malik[2], Inci Yildirim[3], Saad B Omer[2]

[1]Aga Khan University, Karachi, Pakistan, [2]UT Southwestern Medical Center, Dallas, TX, United States, [3]Yale School of Medicine, New Haven, CT, United States

Pakistan bears a high burden of under-five mortality, yet accurate cause-of-death data remain limited due to incomplete civil registration and reliance on low-specificity verbal autopsies. The Child Health and Mortality Prevention Surveillance (CHAMPS) network addresses this gap using minimally invasive tissue sampling (MITS) and expert review. This report presents an analysis of the first 150 cases for which Minimally Invasive Tissue Sampling (MITS) was successfully consented to and performed. While the broader surveillance remains active, the findings detailed here represent a specific cohort of community-based deaths occurring between August 2023 and November 2025. MITS procedures were performed via a mobile van designed for use in informal settlements while respecting religious and cultural norms. A multidisciplinary Determination of Cause of Death (DeCoDe) panel assigned ICD-11-coded causes using clinical data, verbal autopsies, histopathology, and molecular diagnostics. Of 1935 notification, 1353 were eligible, 548 were not eligible and 150 cases were consented for MITS, including 31.3% of stillbirths, 49.3% of neonates, 13.3% of post-neonates, and 6.0% of children aged 1-5 years. Further, 70.5% of deaths occurred in facilities, and 29.5% were home-based deaths. In addition, 70% of post-neonatal deaths occurred at home. Community engagement was vital, with 56.6% of alerts coming from healthcare providers and 18.6% from community volunteers. Undernutrition was widespread (94.7% underweight; median BMI 10.8). Median time from death to MITS was 3.21 hours. Leading underlying causes were intrauterine hypoxia (n=19), prematurity (n=17), and sepsis (n=15); common immediate causes were sepsis (n=23) and pneumonia (n=16). Overall, MIT's surveillance identifies the causes of death in both hospital and home settings, with the primary reason being malnutrition, infection, and delivery process complications. The CHAMPS Pakistan model demonstrates a feasible, culturally sensitive approach to generating actionable mortality data in resource-limited, high-burden settings.

6994

HEALTH-SEEKING PATHWAYS AMONG UNDER-FIVE MORTALITY CASES IN URBAN SETTING: FINDINGS FROM CHILD HEALTH AND MORTALITY PREVENTION SURVEILLANCE IN PAKISTAN

Aiman Mahmood Khamisani[1], Raheel Allana[1], Mahder Tracy[2], Sajid Shah[1], Zoyia Hassan[3], Fauzia Aman Malik[4], Narjis Rizvi[1], Abdul Momin Kazi[5], Saad Omer[6]

[1]Aga Khan University, Karachi, Pakistan, [2]Dallas, TX, United States, [3]Karachi, SD, Pakistan, [4]UT Southwestern Medical Center, Karachi, TX, United States, [5]Aga Khan University, Karachi, SD, Pakistan, [6]Yale University

Pakistan has the highest under-five mortality rate (U5MR) in South Asia, two-thirds of these deaths occur in neonatal period. Despite known medical and socio-economic factors, evidence on health-seeking pathways and provider choices before death in low-income urban settings remains limited. Understanding these pathways is critical to reduce preventable child deaths. This study assessed initial care choices (formal versus alternative provider), and mapped sequence of healthcare interactions from symptom onset to death. A retrospective analysis was conducted using Child Health and Mortality Prevention Surveillance (CHAMPS) verbal autopsy data, Pakistan. The study included 267 under-five deaths (August 2023-December 2024), excluding stillbirths, sudden deaths, and incomplete verbal autopsies. Variables included type of first healthcare contact and sequences of care across settings (private clinic, private hospitals, government hospitals, alternative provider and home). Cases were stratified by age group and verbal autopsy narratives were reviewed to code the place of illness onset, care-seeking steps, and place of death. These health-seeking pathway steps (HSPS) were sequenced (up to 11 steps) and visualized using Sankey diagrams. Of the 267 cases, 51% were neonates, 31% infants, and 18% children. Most caregivers sought care from the formal care providers (83%), mainly from private facilities; 13% consulted alternative providers and 4% sought no care. The Sankey diagram illustrated that the pathways were highly fragmented, with frequent transitions between home and private facilities. Early mortality was high among neonates (60% deaths by HSPS-2), whereas most infant and child deaths occurred around HSPS-6. Hospitals accounted for the largest proportion of deaths and deaths on route were notable among infants (n=15). Recurrent oscillations between care points suggest weak referral systems and delays in care. The study highlights gaps in continuity of care and suggests improving caregiver awareness, referral linkages, emergency transport, and pediatric critical care to reduce preventable under-five mortality.

6995

LEVERAGING REAL-WORLD DATA CAN STRENGTHEN SURVEILLANCE AND HIV CARE SYSTEMS IN BURKINA FASO BY IMPROVING POSTNATAL FOLLOW-UP, COMMUNITY KNOWLEDGE, AND RELIABLE ACCESS TO ESSENTIAL RESOURCES

Fati Kirakoya-Samadoulougou[1], Sekou Samadoulougou[1], Lucresse Corine Fassinou[1], Blaise Barche[1], Hamidou Sanou[1], Wedminere Noëlie Zoungrana-Yameogo[2], Maxime Koiné Drabo[3], Nicolas Meda[4]

[1]Université Libre de Bruxelles, Brussels, Belgium, [2]Département de santé publique, Centre hospitalier universitaire de Tengandogo, Ouagadougou, Burkina Faso, [3]Institut de recherche en sciences de la santé, Ouagadougou, Burkina Faso, [4]UFR Sciences de la Santé, Université Joseph Ki-Zerbo, Ouagadougou, Burkina Faso

Achieving the 2030 target for eliminating vertical transmission of HIV requires resilient approaches that maximize the use of existing data. This study leveraged multiple real-world data sources to assess prevalence, temporal trends in the continuum of HIV care in Burkina Faso and identify determinants influencing prenatal and postnatal services. A multi-level approach used routine data from the District Health Information System version 2 (2013-2024), service availability and readiness assessments (2012-2018), the 2021 Demographic and Health Survey, and the 2019-2020 national ART adherence survey. Data triangulation using DHIS2, PROMISE project data, UNAIDS reports, and DHS indicators improved data validity. Prevalence, service utilization trends, and determinants at individual, community, and structural levels were examined, with socio-demographic determinants ranked using machine learning. HIV testing during antenatal care increased from 82.3% in 2013 to 95.4% in 2024, and ART initiation among HIV-positive pregnant women from 41.5% to 97.2%. However, only half of facilities and six in ten had readiness to deliver provider-initiated HIV testing and counseling in 2018. Three regions (Cascades, Centre-Est, Est) showed low utilization and limited readiness. Data triangulation showed strong convergence for prenatal indicators but discrepancies for postnatal indicators. Persistent gaps included suboptimal antenatal care adherence (79.6%) and limited postnatal follow-up. Early infant diagnosis increased modestly (44.1% in 2013 to 50.3% in 2024), while final testing at 18 months rose from 21% to 24.6%. Among women on ART, 86.6% were adherent. Determinants included individual factors (HIV knowledge, education, occupation, maternal age ≥25), community factors (urban residence, media access), and structural factors (stock-outs, workforce shortages). Leveraging real-world data can strengthen surveillance and HIV care systems in Burkina Faso by improving postnatal follow-up, community knowledge, and reliable access to essential resources.

6996

MATERNAL SEXUALLY TRANSMITTED INFECTIONS AND RISK OF ADVERSE BIRTH OUTCOMES IN A PROSPECTIVE COHORT OF PREGNANT WOMEN IN GHANA

Dennis Gyasi Konadu[1], Veronica Agyemang[1], Alex Kwame Owusu-ofori[2], Dennis Adu-Gyasi[1], Irene. Azindow[1], Stephaney Gyaase[1], Richard Tetteh[1], Eliezer Lartey[1], David Kwame Dosoo[1], Kwaku Poku Asante[1]

[1]Kintampo Health Research Centre, Kintampo, Ghana, [2]Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

Sexually transmitted infections (STIs) during pregnancy are associated with adverse maternal and neonatal outcomes, yet their burden and impact remain understudied in many sub-Saharan African settings. We assessed the prevalence of common STIs and their association with adverse birth outcomes among pregnant women in rural Ghana. Pregnant women (1,033) aged <20 gestation weeks in the Kintampo North and South districts of Ghana were consented and enrolled into a prospective cohort study. Vaginal swab samples were collected for the detection of Chlamydia and gonorrhea by GeneXpert (Cepheid); HIV and syphilis by rapid diagnostic test (Abbott Bioline HIV/Syphilis Duo). A composite STI variable was defined as the presence of any of the four infections. Participants were followed through delivery. Outcomes included preterm birth (<37 weeks) and low birthweight (<2500 g). Logistic regression models were used to assess associations between STIs and outcomes, adjusting for maternal age, parity, gestational age at enrolment, and socioeconomic status (SES). Prevalence of chlamydia, HIV, gonorrhoea, and syphilis were 8.6%, 2.0%, 1.6%, and 0.6%, respectively. Overall, 5.6% of women delivered preterm and 12.8% had babies with low birthweight. In multivariable analysis, composite STI variable was not associated with preterm birth (OR: 0.96; 95% CI: 0.34–2.72) or low birthweight (OR: 0.74; 95% CI: 0.37–1.49). Younger maternal age was independently associated with higher odds of STI, with women aged 21–29 years (OR: 0.44; 95% CI: 0.27–0.72) and ≥30 years (OR: 0.22; 95% CI: 0.13–0.40) less likely to have an STI compared to those <20 years. Socioeconomic status showed a non-significant trend, with women in the lowest wealth category having lower odds of STI. STIs were relatively common, particularly among younger women, but were not associated with adverse birth outcomes. Early screening and targeted prevention remain essential.

6997

MEASLES-ASSOCIATED MORTALITY AMONG CHILDREN UNDER FIVE IN PAKISTAN: EVIDENCE FROM CHAMPS SURVEILLANCE 2023-2025

Raheel Allana[1], Mahder Tracey[2], Aiman Mahmood Khamisani[1], Zoyia Hassan[1], Sajid Shah[1], Zahra Hoodbhoy[1], Shabina Ariff[1], Fauzia Aman Malik[2], Saad B. Omer[2], Abdul Momin Kazi[1]

[1]Aga Khan University, Karachi, Pakistan, [2]UT Southwestern Medical Center, Dallas, TX, United States

Measles continues to be a major cause of preventable childhood mortality in low-resource settings such as Pakistan, even though a safe and effective vaccine is available. This study aimed to quantify measles-attributable mortality and characterize the clinical and epidemiological features of fatal cases to inform targeted public health interventions. We analyzed mortality surveillance data collected through the Child Health and Mortality Prevention Surveillance (CHAMPS) network in Pakistan from August 2023 to November 2025. Causes of death were assigned by a multidisciplinary panel using clinical records, verbal autopsies, and minimally invasive tissue sampling (MITS). Population denominators were derived from an integrated Health and Demographic Surveillance System (HDSS). A total of 74 measles-associated deaths were identified among 828 deaths in children under five years of age. Of these, 62 (84%) occurred in vaccine-eligible children aged ≥9 months, while 8 occurred in children aged 6-8 months and 3 in those aged 2-5 months. Among age-eligible children, only 5 (8%) had received any measles vaccination (4 with two doses and 1 with a single dose). Deaths occurred in both community and facility settings: 24 in community, 44 in facility, and the remainder during transport. Common clinical presentations included measles with pneumonia (n=24), measles with other complications (n=18), meningitis (n=3), and uncomplicated measles (n=29). Within the HDSS population, 16,570 of 36,049 vaccine-eligible children (45.9%) had received at least one measles vaccine dose. Measles remains a substantial contributor to under-five mortality in Pakistan, disproportionately affecting unvaccinated, age-eligible children. The high proportion of deaths occurring in a community and among children not yet vaccinated highlights critical gaps in immunization coverage and timely access to care. Strengthening routine immunization services and implementing targeted strategies to reach under-immunized populations are urgently needed to reduce measles-related mortality.

6998

HOSPITAL LENGTH OF STAY AND MORTALITY IN CHILDREN WITH SEVERE ACUTE MALNUTRITION AND TUBERCULOSIS

Ruth Belay[1], Tania Thomas[2], Domitila Augustino[3], Paulo Mejan[3], Joshua Mungi[3], Restituta Mosha[3], Mariamu Temu[3], Kelvin Msoka[3], Taylor Payne[4], Simeon Kusulla[3], Ghassan Ilaiwy[2], Godfrey Guga[3], Esto Mduma[3], Saning'o Saruni Lukumay[5]

[1]University of Virginia School of Medicine, Charlottesville, VA, United States, [2]University of Virginia, Charlottesville, VA, United States, [3]Haydom Lutheran Hospital, United Republic of Tanzania, [4]University of Virginia, Charlottesville, Charlottesville, VA, United States, [5]Haydom Lutheran Hospital, Haydom, United Republic of Tanzania

Childhood undernutrition remains a significant driver of morbidity, affecting nearly 12% of children under 5 years of age in Tanzania. Of particular concern is how undernutrition renders the body vulnerable to infectious diseases including tuberculosis (TB). This study aimed to evaluate hospital length of stay and in-hospital mortality among children with severe acute malnutrition (SAM) and TB. We hypothesized that concomitant SAM and TB is associated with longer hospitalization and increased in-hospital mortality compared to having either diagnosis alone. A single center study was conducted in rural Tanzania including children <10 years prospectively admitted with SAM or fever/cough and evaluated for TB through stool testing. This analysis included those who had a discharge diagnosis of SAM and/or TB. SAM was defined by a weight-for-height z-score (WHZ) 3 standard deviations below the mean, a mid-upper arm circumference (MUAC) < 115 mm, or the presence of pedal edema. Our primary outcomes included length of stay and in-hospital mortality. We used simple frequencies to describe age, gender, breastfeeding status, duration of illness, and anthropometrics. Non-parametric tests were used to compare length of stay between diagnosis groups; Chi-square was used to compare in-hospital mortality. We analyzed data from 83 children, including n=63 with SAM (median age: 1.37 IQR: 0.90-2.20), n=7 with TB (median age: 1.98 IQR: 1.36-4.36), and n=13 (median age: 0.78 IQR: 0.67-1.29) with both SAM and TB (p=NS between groups). Length of stay was a median of 9 days (IQR: 6-11) for children with SAM, 7 days (IQR: 6-10) for those with TB, and 11 days (IQR: 6.5-17.5) for those with TB and SAM; there was no significant difference between groups (p=0.24). In hospital mortality occurred in 6 children with SAM (9%), and none in the other groups (p=0.36). Concomitant SAM and TB were not associated with a longer hospital stay in this cohort; we plan to look at food insecurity scores, seasonality and other variables that might affect mortality in SAM.

6999

PERFORMANCE OF COMMUNITY HEALTH WORKERS AND ASSOCIATED FACTORS IN THE NORTH AND FAR NORTH REGIONS OF CAMEROON

Wirngo Mohamadu Suiru

Jhpiego, Yaounde, Cameroon

Community Health Workers (CHWs) are vital to primary healthcare in low- and middle-income countries. In Cameroon, about 10,000 CHWs deliver prevention, health promotion, case management, and surveillance services in underserved areas. However, performance varies due to sociodemographic and health system factors, with education, residence, and experience as key determinants, and strong support mechanisms (training, supervision, incentives) improving outcomes. However, Cameroon's CHW system remains only partially functional, with persistent gaps in support and limited evidence from heavily burdened and insecure northern regions. This study assessed CHW performance and associated factors in the North and Far North regions using a cross-sectional analytical design among 2,297 CHWs (59.3% Far North; 40.7% North). Most CHWs were aged 25-44 years (73.2%), had secondary education (51.3%), and were mainly farmers (76.1%), most were male (81.1%). Overall, 41.3% demonstrated good performance, while 58.7% had low performance. Performance was low for consultations (25.9%), referrals (26.0%), malaria testing (26.0%), and diarrhea management (26.9%), but moderate for uncomplicated malaria treatment (52.8%), home visits carried out (54%), and high for reporting (93.9%). Good performance was associated with residence in the Far North (aOR=1.53; 95% CI: 1.27-1.84), secondary education (aOR=1.28; 95% CI: 1.06-1.56), and being a teacher (aOR=4.99; 95% CI: 2.45-10.15), while sex, age, and experience were not significant. The low performance observed by 58% highlights the need to strengthen selection criteria and technical support for CHWs. This performance remains suboptimal particularly for essential clinical services, highlighting the need to strengthen training, supervision, and recruitment criteria to improve effectiveness.

7000

IMPACT OF STRENGTHENING THE INTEGRATED MANAGEMENT OF CHILDHOOD ILLNESS (IMCI) AT THE COMMUNITY LEVEL ON THERAPEUTIC COVERAGE IN BURKINA FASO: A MIXED-METHODS EVALUATION CONDUCTED IN THREE REGIONS

Andre Kone[1], Yacouba Ouedraogo[1], Issa Ouedraogo[1], Yacouba Zanre[1], Yacouba Belem[1], BADOLO OUSMANE[2], Mahamadi Cisse[3], Eva Lathrop[4]

[1]Jhpiego, Ouagadougou, Burkina Faso, [2]Jhpiego, OUAGADOUGOU, Burkina Faso, [3]DSF, Ouagadougou, Burkina Faso, [4]Jhpiego, Baltimore, MD, United States

Under-five mortality in Burkina Faso remains a major public health concern. To help reduce neonatal mortality particularly in the regions of Djoro(12 p.

1,000), which recorded neonatal mortality rates above the national average (8 p.1,000) in 2024, the Government of Burkina Faso, with support from the United States Government, trained and equipped 3,335 community health workers in the cIMCI. This initiative aimed to improve the quality and coverage of essential child health services by bringing life-saving care closer to rural communities. After three years of implementation, this evaluation seeks to draw lessons, promote adaptive management, and inform future decision-making by stakeholders involved in cIMCI implementation. A mixed-methods approach was employed for this evaluation, combining a literature review, secondary quantitative data analysis, and qualitative data collection. Quantitative data on community health worker activities were extracted from the ENDOS system. In addition, semi-structured interviews were conducted with a purposive sample of 30 community health workers, selected to represent the diversity of implementation contexts, including rural settings and different health zones. Findings from the quantitative and qualitative analyses were triangulated with the literature review to identify key challenges and inform actionable recommendations. ENDOS data show a substantial increase in childhood illness consultations between 2022 and 2024, with malaria cases tripling (from 39,908 to 122,970), diarrhoea cases from 0 to 10,200 and cough cases from 0 to 121,181. Treatment quality remained high, with 94% of malaria cases laboratory confirmed, 90% of cough cases treated with amoxicillin, and 89% of diarrhoea cases managed with ORS and zinc. These results suggest that strengthening community health worker capacity and providing essential equipment improved the management of underfive illnesses at the community level. The challenges identified through the analysis will inform efforts to further strengthen cIMCI implementation in Burkina Faso

7001

ASSESSMENT OF COMMUNITY LEVEL DATA QUALITY FOR INTEGRATED COMMUNITY CASE MANAGEMENT AMONG UNDER-FIVE CHILDREN IN SIX DISTRICTS IN ZAMBIA

Prudence M. Malama[1], Christopher Lungu[1], Todd Jennings[1], Martin Mwansa[2], Eric Liswaniso[3], Dingase Phiri[1], Benson Mwelwa[1], Chrispine Syamupa[2], Ian Mwanza[2], Donald Mukumbuta[4]

[1]PATH, Lusaka, Zambia, [2]World Vision Zambia, Lusaka, Zambia, [3]Malaria Partners Zambia, Lusaka, Zambia, [4]National Malaria Elimination Centre - Ministry of Health, Lusaka, Zambia

The Zambia Ministry of Health (MoH) implements Integrated Community Case Management (iCCM) as a key strategy to reduce morbidity and mortality among children under five. In iCCM, Community Health Workers (CHWs) are trained to assess, classify, treat, refer, and report on childhood illnesses using standardized tools and registers. The Rotary Healthy Communities Challenge (RHCC) aims to expand community-level care in six districts using CHWs to diagnose and treat malaria, pneumonia, and diarrhoea, and to refer for malnutrition. However, limited routine verification of community data has constrained effective monitoring. To address this gap, RHCC adapted Zambia's malaria Routine Data Quality Audit (RDQA) tool to include community level iCCM data for malaria, pneumonia, diarrhoea, and malnutrition. A cross-sectional RDQA will be conducted in May-June 2026 using this MoH-aligned RDQA tool. CHW iCCM registers will be reviewed and compared to aggregated monthly reports submitted to the national District Health Information Software (DHIS2) system. The audit will assess under-five indicators for malaria rapid diagnostic testing and treatment with ACTs, pneumonia assessment and amoxicillin treatment, diarrhoea management with ORS and zinc, and identification and referral of malnutrition. Reporting completeness, accuracy by data element and reporting month, and consistency between source documents and reported data elements will be evaluated. Validation will occur in real time at community and facility levels, with discrepancies documented and reviewed with CHWs and their supervisors. The RDQA will generate baseline evidence on community level iCCM data quality and identify system gaps affecting accurate reporting following CHW deployment. Findings will inform data quality improvement actions, supportive supervision, and monitoring frameworks aligned with project goals to reduce morbidity and mortality of malaria, pneumonia, and diarrhoea in under-five children.

7002

OPTIMIZING COMMUNITY CARE SITE EXPANSION USING GEOSPATIAL CCM BENCHMARKING IN KASAI PROVINCE, DEMOCRATIC REPUBLIC OF THE CONGO

Cedric A. N'konzi[1], Justin Millar[2], Remy Ngoy[1], Travis Porter[3], Todd Jennings[4], Welo Placide[5]

[1]PATH, Kinshasa, Democratic Republic of the Congo, [2]PATH, Seminole, FL, United States, [3]PATH, Seattle, WA, United States, [4]PATH, Lusaka, Zambia, [5]Ministry of Health DRC, Kinshasa, Democratic Republic of the Congo

In the Democratic Republic of the Congo (DRC), volunteer-run Community Care Sites (CCSs) provide first-line testing, treatment, and referral for malaria, diarrhea, and pneumonia, and referral for severe illness, acute malnutrition, and pediatric tuberculosis. CCSs are designed to serve populations living in hard-to-reach areas—typically where access to formal health facilities is limited. Despite an extensive CCS network throughout DRC, significant gaps in access to services persist, and the national Community Care Sites Extension Plan has called for use of geospatial approaches to maximize equitable access to care. In 2025, PATH conducted a series of co-creation workshops with national iCCM stakeholders to develop a geospatial modeling approach to address priority expansion planning needs. The resultant model incorporated travel time "friction" surfaces, population density, and existing care site data to map hard-to-reach populations greater than sixty minutes travel time from a provider and estimate the number of additional CCS needed to meet service coverage benchmarks. Outputs were built into an interactive explorer tool that allowed users to visualize geospatial gaps and estimate CCS needs within individual Health Zones. In collaboration with the Rotary Healthy Community Challenge (RHCC) project, this tool was applied within 18 Health Zones of Kasai Province to estimate the total population within hard-to-reach areas and the number of additional CCSs needed to address service access gaps. Care site benchmarks were then used to prioritize expansion in health zones with the largest access gaps, align CCS placement with disease burden and population density, and reconcile coverage gaps with previous partner service point data. This experience has demonstrated how geospatial modeling can be used to operationalize community-based service provision expansion strategies through a data-driven approach, enabling targeted placement of services where unmet need is greatest, and highlighting the increasing importance of such approaches for optimizing the use of limited global health resources.

7003

BASELINE ASSESSMENT OF THE SIX-WEEK ATTACHMENT PERIOD TO STRENGTHEN COMMUNITY HEALTH WORKER SKILLS TO ASSESS MALARIA, PNEUMONIA, AND DIARRHEA IN ZAMBIA

Christopher Lungu[1], Martin Mwansa[2], Prudence Malama[1], Donald Mukumbuta[3], Todd Jennings[1], Chrispine Syamupa[2], Ian Mwanza[2]

[1]PATH, Lusaka, Zambia, [2]World Vision Zambia, Lusaka, Zambia, [3]National Malaria Elimination Centre - Ministry of Health, Lusaka, Zambia

Integrated Community Case Management (iCCM) is a key Zambia Ministry of Health (MoH) strategy for reducing morbidity and mortality among underfive children through timely diagnosis and treatment at community level. In 2025, MoH in partnership with PATH, Rotary International, and World Vision, began implementing the Rotary Healthy Communities Challenge (RHCC), an iCCM project, in six districts. Zambia uses a 6-week attachment period at health facility level following Community Health Worker (CHW) trainings in malaria community case management; this period is for practicals between training and deployment. The RHCC project added greater structure to the attachment period, introducing a checklist for the CHW supervisor who observes patient interactions for all iCCM conditions, provides mentorship, and tracks progress. A baseline assessment of the attachment period was undertaken in five districts. In all, a sample of 40 health facilities and 462 CHWs took part in the exercise to assess the readiness of CHWs to implement iCCM in their communities.

An assessment guide covered general progress by the CHW and the level of supervision provided; weekly attendance and task summaries; case management competency (malaria, pneumonia, and diarrhea); communication, and clinical judgement. 83% of CHWs correctly used the sick child recording form while 65% demonstrated accurate identification of danger signs and respiratory rate counting. Procedures were largely correct for RDT use with appropriate referrals made. However, there were gaps with the administration of amoxicillin. On registers and data management, 83% of registers were correctly filled with 70% signed by supervisors; 85% data consistency was observed, indicating reliable reporting. To strengthen clinical competency, it is recommended to provide refresher training and mentorship focused on danger sign recognition, respiratory rate counting, and correct amoxicillin administration; reinforcing supervision practices; and ensuring the CHW supervisors at health facilities consistently record tasks and sign-off on logbooks.

7004

PREVALENCE, TIMING, AND RISK FACTORS OF MATERNAL MORTALITY: EVIDENCE FROM THE CHILD HEALTH AND MORTALITY PREVENTION SURVEILLANCE NETWORK

Jonathan Andrew Muir[1], Shiyi He[1], Navit T. Salzberg[1], Kasthuri Sivalogan[1], Kyu Han Lee[1], Yasir Abdullahi[2], Nega Assefa[2], Cynthia Gayle Whitney[3]

[1]The Taskforce for Global Health, Decatur, GA, United States, [2]Haraghe Health Research, Haramaya, Ethiopia, [3]Emory University, Atlanta, GA, United States

Maternal mortality remains unacceptably high in many resource-limited countries, particularly in sub-Saharan Africa (SSA), despite global commitments to the Sustainable Development Goal (SDG) target of <70 maternal deaths per 100,000 live births by 2030. Approximately 260,000 maternal deaths occur annually, with 92% occurring in resource-limited countries. Sub-Saharan Africa (SSA) continues to bear the highest burden of preventable maternal deaths, reflecting the combined effects of poverty, inequitable access to care, weak health systems, and entrenched gender inequalities. To better inform efforts to improve maternal health and survival and thereby attain the SDG target, we investigated the prevalence and associated risk factors of maternal mortality within the data reported through Pregnancy Surveillance (PS) activities of the Child Health and Mortality Prevention Surveillance (CHAMPS) network, which enrolls pregnant women and women who recently delivered and collects data from the prenatal, delivery, and post-natal period. At enrollment, information on maternal sociodemographic characteristics, obstetric history, and medical conditions are collected; pregnancy, delivery, neonatal and maternal outcomes are collected during follow up visits. We calculated descriptive statistics for maternal demographics, pregnancy characteristics and outcomes, and maternal mortality ratios; we analyzed associations between maternal mortality and risk factors via logistic regression. Since its inception, CHAMPS PS has enrolled 117,580 women, recorded 95,761 live births, and documented 180 maternal deaths across all sites. Preliminary results suggest maternal mortality ratios (MMR), defined as maternal deaths per 100,000 live births, higher than the SDG targets in Bangladesh (288.9 MMR), Ethiopia (174.2 MMR), Kenya (128.2 MMR), Mozambique (117.9 MMR), Nigeria - Bauchi (421.0 MMR), Nigeria – Cross River (200.8 MMR), and Sierra Leone (327.7 MMR).

7005

EXPLORING HEALTHCARE AND COMMUNITY HEALTH WORKERS' PERSPECTIVES ON AN INTEGRATED SERVICE DELIVERY FOR RTS,S MALARIA VACCINATION IN LIBERIA

Lisa Jesslyn Williams, Wahdae-Mai Harmon-Gray, Joseph Sieka

University of Liberia School of Public Health, Monrovia, Liberia

From September to November 2025, an integrated service delivery intervention was implemented across four southeastern counties in Liberia to improve the uptake of the RTS, S malaria vaccine, particularly the fourth dose. The interventions included refresher training for health and community health workers, triage of child road-to-health booklets at facility entry points, community health worker-led defaulter-tracing and referral, and co-delivery of vitamin A, deworming, and insecticide-treated nets alongside the fourth RTS,S dose. To evaluate perceptions of this approach, 37 in-depth interviews were conducted with health and community health workers across Maryland, Grand Kru, Grand Gedeh, and Rivercess counties. Audio-recorded interviews were transcribed and analyzed using thematic analysis. Participants perceived that the integrated service delivery approach improved vaccination uptake and service efficiency, particularly through active defaulter tracing and same-day service delivery. Health workers reported increased completion of the fourth RTS,S dose and noted perceived reductions in severe malaria cases. The co-delivery of services was viewed positively, with participants describing improved convenience for caregivers through access to multiple services in a single visit. Community health workers described defaulter tracing as effective, though challenges such as delayed defaulter lists, network constraints, and competing caregiver priorities limited follow-up. Healthcare workers also reported increased workload, higher patient flow, and additional documentation requirements as key operational challenges. Participants also noted that system-level gaps, including a lack of standardized tools for recording deworming and insecticide-treated net distribution, as well as commodity stockouts and coordination challenges, affected the consistency of service delivery. Despite these difficulties, the intervention was widely regarded as feasible and beneficial, highlighting the need to strengthen health system support to sustain and scale integrated vaccination strategies.

7006

LIVED EXPERIENCES OF CONTRACEPTION USE AMONG HIV-POSITIVE WOMEN TREATED FOR CERVICAL DYSPLASIA IN WESTERN KENYA

Joan Chepngeno[1], Susan Cu-Uvin[1], Elkanah O. Omenge[2]

[1]Brown University, Providence, RI, United States, [2]The Aga Khan University, Nairobi, Kenya

Women aged 15-24 years accounted for almost a third of new HIV infections in East and Southern Africa in 2023. Women living with HIV (WLHIV) are six times more likely to develop cervical cancer compared to HIV-negative women. Cervical dysplasia, a precursor to cervical cancer, is typically managed with excisional procedures such as the Loop Electrosurgical Excision Procedure (LEEP), which may increase the risk of adverse obstetric outcomes. Family planning is essential for this population to prevent vertical HIV transmission, reduce unintended or mistimed pregnancies, and promote overall reproductive health. However, contraceptive use remains low, with nearly 40% of pregnancies reported as unplanned or mistimed within this demographic. We sought to identify family planning needs and examine perspectives and decision-making processes around contraceptive use among women living with HIV who had been treated for cervical dysplasia in Western Kenya. Semi-structured, in-depth interviews were conducted with 17 WLHIV who had a history of contraceptive use and prior treatment for cervical dysplasia, as well as 8 healthcare providers at Moi Teaching and Referral Hospital. Transcripts were translated from Swahili to English and analyzed thematically using MAXQDA Analytics Pro (version 24.2.0). Participants demonstrated familiarity with multiple contraceptive methods, although misconceptions regarding implants and intrauterine devices were prevalent. Contraceptive decision-making was shaped by interpersonal relationships, cultural norms, and family planning counseling. While some women expressed a desire for future fertility, many reported that they had received insufficient information about the options that were available and appropriate for them. Barriers to sustained contraceptive use included side effects, limited availability of preferred methods, and concerns about potential interactions between antiretroviral therapy and hormonal contraceptives. There is need for better integration of family planning services in HIV care and cervical screening clinics to optimize contraceptive access and uptake for WLHIV.

7007

VERBAL AUTOPSY FINDINGS: ALMOST HALF OF UNDER-5 DEATHS IN RURAL GAMBIA OCCUR AT HOME.

Isaac Osei[1], Baleng Mahama Wutor[2], Galega Lobga[1], Williams Adefila[1], Grant A. Mackenzie[3]

[1]MRC Unit The Gambia At The London School Of Hygiene And Tropical Med, Banjul, Gambia, [2]Rollings School of Public Health, Emory University, Atlanta, GA, United States, [3]MRC Unit, The Gambia, Basse, Gambia

In rural Gambia, childhood deaths outside healthcare facilities often go unreported due to weak Civil and Vital Registration Systems, and information on their scale and causes remains limited. Verbal autopsy (VA) is a vital method for establishing the causes of death in communities with limited healthcare access. We assessed the causes of mortality among children under-5 years in rural Gambian households. We used the 2016 WHO questionnaire to perform VAs for all children under-5 deaths in the Basse and Fuladu West HDSS in rural Gambia, from September 2019 to December 2023. Trained personnel conducted VAs electronically, and causes of death were determined through the physician-certified VA method. The cause-specific mortality proportions were calculated, and relevant contextual factors were evaluated. The VA survey identified 1,455 deaths among children under-5 during the study period. Most of these deaths occurred among neonates (n=599 [41%]), followed by ages 1–11 months (n=382 [26%]). There was a balanced number of male and female deaths. The three leading primary causes of death were acute respiratory tract infections, including pneumonia (17%), sepsis (14%), and diarrheal diseases (13%). Birth asphyxia, neonatal sepsis, and prematurity/low birthweight were the main causes among neonates. For children aged 1–11 months and 1–4 years, acute respiratory infections, including pneumonia and diarrheal diseases, were the predominant causes, accounting for 34% and 26%, respectively. Severe malnutrition (23%) and prematurity/low birth weight (11%) were the leading underlying causes across all age groups. Nearly half of the deaths occurred at home (44%). Although 55% of caregivers recognised that the deceased required medical attention and 92% of families lived within two hours of a health facility, care was not sought outside the home in 42% of cases. In 28% of cases, the cost of medical treatment was a barrier. Almost half of the deaths of children under-5 in rural Gambia occur at home. These findings underline the urgent need for community-based interventions to reduce home deaths among children under five in rural Gambia.

7008

PREGNANCY SURVEILLANCE (PS) IN CHILD HEALTH AND MORTALITY PREVENTION SURVEILLANCE NETWORK (CHAMPS): METHODS AND FINDINGS

Nega Assefa[1], Yasir Abdullahi[1], Jonathan Andrew Muir[2], Lola Madrid[3], Mohammed Jemal[1], Shams El Arifeen[4], Emily S. Gurley[5], Mohammad Zahid Hossain[6], Atique Iqbal Chowdhury[7], Asraful Alam[8], Hellen Muttai[9], Aggrey Kitiezo Igunza[10], Dickson Gethi[9], George Aol[9], Karen Kotloff[11], Samba O. Sow[12], Adama Mamby Keita[12], Uduma Uma Onwuchekwa[13], Felizarda Elisa Amosse Nhacolo[14], Ariel Quingue Nhacolo[15], Inaceo Mandomando[16], Quique Bassat[17], Ikechukwu Ogbuanu[18], Soter Ameh[19], Shabir Madhi[20], Ziyaad Dangor[20], Bongani Ntimani[20], Siobhan Johnstone[20], Portia Mutevedzi[2], Chelsea Cole[2], Anthony Scott[1], Cynthia Gayle Whitney[2]

[1]Haraghe Health Research, Haramaya, Ethiopia, [2]The Taskforce for Global Health, Decatur, GA, United States, [3]London School of Hygiene and Tropical Medicine (LSHTM), Harar, Ethiopia, [4]International Centre for Diarrheal Disease Research, Bangladesh (icddr,b), Dhaka, Bangladesh, [5]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [6]icddr,b, Dhaka, Bangladesh, [7]icddr,b, Dhaka, 13, Bangladesh, [8]ICDDRB, Dhaka, Bangladesh, [9]Kenya Medical Research Institute (KEMRI), Kisumu, Kenya, [10]KEMRI - CGHR, Kisumu, Kenya, [11]Univ of Maryland School of Medicine, Baltimore, MD, United States, [12]Center for Vaccine Development Mali, Bamako, Mali, [13]Centre for Vaccine Development - Mali, Bamako, Mali, [14]Manhica health researcher center, Vila Da Manhica, Mozambique, [15]Manhica Health Research Center, Manhica, L, Mozambique, [16]Centro de Investigagao Em Saude Da Manhoa, Maputo, United States, [17]ISGlobal, Barcelona, Spain, [18]CHAMPS, Makeni, Sierra Leone, [19]Freetown, W, Sierra Leone, [20]Medical Research Council, and Infectious Diseases Analytics Research Unit (VIDA), Soweto, South Africa

Child Health and Mortality Prevention Surveillance (CHAMPS) is designed to determine causes of stillbirth and deaths in children <5 years in high-mortality settings. Because most CHAMPS-enrolled deaths are identified during the perinatal period, we launched pregnancy surveillance to better understand maternal care in high child-mortality settings. Pregnant women were enrolled during home visits or prenatal care from catchment populations in eight countries (Bangladesh, Ethiopia, Kenya, Mali, Mozambique, Nigeria – Cross River, Sierra Leone, and South Africa) and followed to determine pregnancy, neonatal, and maternal outcomes. At enrollment, data on maternal sociodemographic characteristics, obstetric history, and medical conditions were collected using REDCap software. Data on maternal and baby healthcare and outcomes are collected for the delivery and through 42 days postpartum. We enrolled 84,013 pregnant women with an attrition rate of 3.0%. Most (83.0%) participants were young (under 30 years of age, with a median age of 25.2), married (91.7%), and had completed up to post-secondary education (66.7%); the median gestational age at enrollment was 21.3 weeks (range 1-42). Most participants had prior pregnancies (70.6%), and the majority (64.1%) gave birth in healthcare settings. However, a considerable proportion of deliveries (18.4%) were unsupervised or unskilled home delivery across sites. Overall, we found high stillbirth (22.9/1000 births, 95 % CI: 21.7 – 26), neonatal mortality (17.6/1000 live births, 95% CI: 16.6 -18.6), and perinatal mortality rates (36.7/1000 live births, 95% CI: 35.3- 38.1), with significant variation ($p< 0.05$) across sites. Our study highlights the gap in early initiation of ANC, the high burden of stillbirths and low birth weight babies, and the continued public health challenge of neonatal mortality rates.

7009

MATERNAL UNDERNUTRITION IMPACTS HUMAN MILK MIRNA EXPRESSION IN THE PHILIPPINES

Nimrit Ahuja[1], Cliff Oduor[1], Veronica Tallo[2], Marianne J. Sagliba[3], Amabelle J. Amoylen[3], Jennifer F. Friedman[4], Jeffrey A. Bailey[1], Meagan A. Barry[5]

[1]Brown University, Providence, RI, United States, [2]Research Institute for Tropical Medicine, Montinlupa, Philippines, [3]Research Institute for Tropical Medicine, Manila, Philippines, [4]Center for International Health Research, Providence, RI, United States, [5]Warren Alpert Medical School of Brown University, Providence, RI, United States

Breastfeeding provides numerous health benefits for infants, likely mediated by milk's bioactive molecules. Human milk is a rich source of miRNA, short non-coding RNA that downregulate post-transcriptional gene expression. Prior studies link maternal obesity to altered miRNA profiles and infant growth, but the impact of maternal underweight status remains unexplored. This study aims to characterize the milk miRNA profile of underweight mothers and their links to infant growth. Pregnant women in Leyte, Philippines were enrolled in a parent study on helminth infection. At 1 month postpartum, milk samples were collected and maternal BMI assessed. Infant growth was tracked for 24 months. In this study, miRNA was extracted from milk samples, sequenced using Illumina NextSeq platform, and reads were mapped to the miRDeep2 database. miRNA profiles of underweight women (BMI < 18.5 kg/m^2, n=4) were compared to normal weight women (18.5–24.9 kg/m^2, n=3). Biologic ramifications were elucidated using the predicted target database microT-CDS and the experimentally validated database Tarbase (DIANA-miRPath v.3.0). Principal Component Analysis revealed distinct separation of the underweight from the normal weight group, suggesting distinct miRNA expression patterns. Fourteen miRNAs were upregulated in underweight samples (FDR < 0.01). Using the predicted target database, there were 89 proposed biological pathways of the upregulated miRNA, 34 of which related to growth, development, and immune function. When cross-referenced to the experimentally validated database, six key pathways emerged: TGF-beta, PI3K-Akt, MAPK, Wnt signaling, Dorso-ventral axis formation, and Progesterone-mediated oocyte maturation. This study is the first to

assess milk miRNA profiles in underweight women, identify key miRNAs that differ from those in normal weight women, and map these to specific biologic pathways. Future directions include assessing milk miRNA profiles in relation to 24-month infant growth. Our study offers novel insight into biological pathways linking maternal nutrition to infant health and may inform targeted nutrition programs.

7010

A SCHOOL-BASED HEALTH EDUCATION PROGRAM IN KENYA DRAMATICALLY INCREASES THE USE OF ORAL REHYDRATION SOLUTION

Ann Messer[1], Nadia N. Tsado[2], Addison Scales[1], Gabriela Lesiv[1]
[1]One Good Turn Global Health, Austin, TX, United States, [2]Lincoln, RI, United States

The use of Oral Rehydration Solution (ORS) is a proven, low-cost, and life-saving response to diarrheal illness that has long remained underutilized. In Sub-Saharan Africa, the rate of ORS use is under 38%. In poor and rural households, challenges to ORS use include lack of both access and knowledge about how to prepare ORS. This abstract evaluates whether educators trained as school health officers (SHOs) can improve ORS utilization and expand community awareness of practical ORS protocols in low-resource school settings in Kenya. An 18-month evaluation was conducted across 43 schools who participated in a multi-component school health training program in extremely vulnerable areas of Kajiado and Nairobi counties. 191 teachers were trained on diarrhea, dehydration, standardized ORS protocols, zinc, and caregiver education. After a demonstration, SHOs were supervised as they made their own ORS using a simple sugar and salt recipe. Participants then drank their home-made ORS together. Schools submitted structured reports at 6, 12, and 18 months post-training, with baseline data collected retrospectively at month six. The increase in use of ORS was highly significant, with a large effect size. Compared to 0% at baseline, at 18 months 78% of schools reported that they administered ORS at school “frequently or always” when a student was dehydrated and/or had diarrhea. 100% of schools had provided ORS at least once, with a minimum of 647 documented uses across the 43 schools over 12 months. Adherence to evidence-based protocols was high, with 67% of schools referencing guidance “frequently or always” at 18 months. SHOs also extended this knowledge to families. Parent education on ORS use increased from 30% of schools at baseline to 97% at 18 months, with over 403 documented education interactions. Educators trained as SHOs enabled the practical, sustained use of ORS while expanding health literacy among students and families. School-based ORS education and delivery represents a feasible and scalable global strategy to improve early dehydration management and dramatically expand the acceptance and utilization of this critically important therapy.

7011

“YES, WE KNOW THE IMPORTANCE OF BIRTH REGISTRATION, BUT HAVE NOT YET REGISTERED...” - EXAMINING FACILITATORS AND BARRIERS TO BIRTH REGISTRATION IN KENYA USING THE THREE-DELAYS MODEL

Michael Otieno Ogutu
KEMRI-Wellcome Trust Research Programme, Nairobi, Kenya

Birth registration, critical for health planning, remains low across sub-Saharan Africa. In Kenya, registration rates have dropped from 83.8% in 2021 to 70.3% in 2024, making it unlikely the country will meet the 2030 universal registration target. This study explored the factors that facilitate and hinder birth registration and was conducted in three socio-demographically diverse Health and Demographic Surveillance Sites in Kenya. We conducted 31 focus-group discussions, 3 small-group discussions, and 12 key-informant interviews with purposively chosen users and providers of civil registration and vital statistics (CRVS) services. We identified the critical drivers and persistent barriers shaping birth registration, highlighting inequalities in access and uptake, applying the three-delay model. The data were analyzed using a framework-analysis approach. The decision to register births was largely driven by immediate needs, particularly school-related requirements. However, many participants delayed seeking registration services due to limited awareness of the requirements, especially among teenage parents/mothers. Additional barriers included the perceived complexity of the process, prior negative experiences, financial constraints, and cultural norms such as patrilineal practices. Accessing registration services also posed challenges, with participants reporting long distances to registration points and associated travel costs. At CRVS offices, several systemic issues contributed to delays, including stockouts of registers, weak document management systems, insufficient printing materials for birth certificates, and delays in submitting completed documents due to a lack of transport for Registration Assistants (RAs). Perceived corruption within the system also hindered timely registration. Enhancing birth registration and certification in Kenya requires attention to various community, systemic, and contextual factors. Key actions to improve access include expanding access via one-stop service centres, digitizing systems, curbing corruption at all levels, and supporting RAs logistics.

7012

PREDICTIVE MAPPING OF THREATS AGAINST HEALTH SERVICES IN A CONTEXT OF HYBRID INSECURITY ACROSS SIX REGIONS OF CAMEROON 2018-2024

Pierrine Andzongo Ngono, Marcel Nwaha, Priscilla Ambany Anya, Clarisse Mapa-Tassou, Basile Yannick Emah, Linda Augustine Juli Esso Endalle Lovet
Ministry of Health of Cameroon, Yaoundé, Cameroon

Health services in Cameroon are increasingly exposed to incidents combining physical attacks, access restrictions, and digital vulnerabilities, threatening continuity of care. The lack of integrated incident analysis limits proactive health-security planning, highlighting the need to identify key determinants and map high-risk facilities. A retrospective study was conducted on 285 health facilities across six regions of Cameroon from 2018 to 2024. Incident records were combined with facility and contextual characteristics. Twenty variables were screened, followed by multivariable logistic regression and a Random Forest model for risk prediction and mapping. Complementary questionnaires were administered to 480 healthcare workers. All procedures adhered to national ethical standards, with full anonymization of data. One hundred and four facilities <36.5%> reported at least one incident. Three factors were independently associated with incident occurrence: proximity <= 4 km to a main road, low cybersecurity level, and high density of armed-group activity. The Random Forest model achieved high accuracy <AUC = 0.90> and effectively predicted high-risk facilities and zones, primarily along major road corridors and areas of instability. Incidents affecting health services arise from intertwined physical, contextual, and digital vulnerabilities. Combining statistical and machine-learning approaches provides a robust tool for anticipating risks and guiding targeted protection and preparedness measures for health facilities.

7013

FROM MOLECULE TO DRUG APPROVAL: LESSONS LEARNED FROM A PUBLIC-PRIVATE PARTNERSHIP IN DEVELOPING A NEW ORAL ANTIBIOTIC AGAINST GONORRHEA

Caeul Lim[1], Carmen Au[2], O’Brien Seamus[2], Drew Lewis[1]
[1]Innoviva Specialty Therapeutics, Waltham, MA, United States, [2]Global Antibiotics R&D Partnership (GARDP)5, Geneva, Switzerland

New antibiotics remain a global health priority, particularly with the rise of antimicrobial drug resistance. Yet progress from discovery to access is often stalled due to the risk of market failure and the complex regulatory pathways for global approval. These barriers disproportionately affect low- and middle-income countries where access to antibacterial therapies is already scarce. Sustainable models to bring new antibiotics to market

require strong partnerships between drug developers and public health driven organizations that agree on innovation and commitment to the cause. Zoliflodacin, a first-in-class, single-dose, oral antibiotic for the treatment of uncomplicated urogenital gonorrhea exemplifies this public-private partnership. This included the Phase 3 trial, the largest trial conducted for uncomplicated gonorrhea spanning 5 countries. Here, we describe the development pathway of zoliflodacin, from early discovery to successful US FDA approval, highlighting the collaboration between the Global Antibiotic Research & Development Partnership (GARDP), the National Institute of Allergy and Infectious Diseases (NIAID) and Innoviva Specialty Therapeutics (IST).We review critical aspects of the partnership, innovative development and access agreements, including sublicensing, financing mechanisms, manufacturing development, execution of non-clinical studies and clinical trials, surveillance, and regulatory strategies. Through joint advisory discussions, the partnership engaged with key global stakeholders to ensure global impact. We outline key lessons, such as the importance of early planning for shared governance structures and global access, and a commitment to generating representative data for populations and regions with the highest disease burden. We discuss the challenges, such as coordinating oversight, capacity building, integrating legacy data, generating real-world evidence, and meeting regulatory expectations. This example offers a replicable blueprint for future public-private partnerships to accelerate antibiotic development and ensure equitable global access.

7014

IMPLEMENTING AND EVALUATING AN INTEGRATED MORBIDITY MANAGEMENT AND DISABILITY PREVENTION (MMDP) INTERVENTION USING COMMUNITY BASED PARTICIPATORY RESEARCH IN NIGERIA

Sunday Isiyaku[1], Martins Imhansoloeva[1], **Suzie Kushianyan Madaki**[2], Shahreen Chowdhury[3], Catherine Kajang[1], Mohammed Liba[1], Anita Gwom[1], Attahiru Aliyu Aliero[4], Celestine Njoku[5], Chukwuma Anyaike[5], Margo Greenwood[6], Laura Dean[3], Ruth Dixon[6]

[1]Sightsavers, Abuja, Nigeria, [2]Celia Scott Weatherhead School of Public Health and Tropical Medicine, Tulane University, NEW ORLEANS, LA, United States, [3]Liverpool School of tropical medicine, Liverpool, United Kingdom, [4]Kebbi State Ministry of Health, Birnin Kebbi, Nigeria, [5]Federal Ministry Of Health, Nigeria, Abuja, Nigeria, [6]Sightsavers, Haywards Heath, United Kingdom

Community-based participatory research (CBPR) has been proven effective in identifying problems and implementing changes grounded in practical knowledge and experience. It uniquely empowers communities and stakeholders through collaborative and equitable problem-solving approaches thereby fostering program ownership. We implemented two morbidity management and disability prevention (MMDP) interventions: a health worker training manual and information, education and communications (IEC) material, co-developed with persons affected with various neglected tropical diseases (NTDs) and frontline health workers (FLHWs). We evaluated the impact of these interventions on access and delivery of integrated NTDs services using CBPR approach and a series of qualitative and quantitative assessments. These included: pre and post-tests and post training evaluation survey with 47 FLHWs to assess knowledge and skills gained; 8 focus group discussions (FGDs) to understand perceptions and key enablers/barriers to implementing the training; narrative case reports with 12 FLHWs to assess knowledge application and skills retention; and 14 FGDs with community participants to validate and gain perceptions on the interventions. Pre, post, and six-week post-tests scores were analyzed using t-tests and one-way ANOVA. Qualitative data were thematically analyzed. Frameworks for training and for validation, guided evaluation of health workers training and IEC materials. FLHW's knowledge and retention significantly improved post-training from baseline and even further six weeks after applying the training to daily routines. Most participants reported the training to be useful and also feeling confident to interact with patients. Emerging sub-themes from FGDs were grouped into four - reaction, learning, behaviour and results, while findings of IEC materials evaluation were summarized by face validity, content validity, construct validity, concurrent validity and relevance. Our findings highlight effectiveness and relevance of the interventions, validating CBPR as a valuable tool for enhancing access and delivery of MMDP services.

7015

PROGRESS AND GAPS IN IMMUNIZATION COVERAGE IN POLIO HIGH-RISK DISTRICTS OF PAKISTAN: A COMPARATIVE ANALYSIS OF HOUSEHOLD SURVEY ROUNDS

Imtiaz Hussain[1], Ahmad Khan[1], Muhammad Umer[1], Sajid Bashir Soofi[2]

[1]Aga Khan University, Karachi, Pakistan, [2]Aga Khan University, Pakistan, Karachi, SD, Pakistan

Monitoring immunization coverage in high-risk geographies is critical for improving equity and program performance. Surveys conducted in Super High-Risk Union Councils (SHRUCs) provide key district-level insights and enable comparison across rounds to track progress and identify persistent service delivery gaps. In 2023 and 2025, two rounds of surveys were done in seven districts of Pakistan using a two-stage stratified cluster sampling design. The surveys covered 39 SHRUCs. In 2025, data were collected from 7,714 children aged 12-23 months and compared with the 2023 findings. Immunization status was classified as fully immunized (FIC), partially immunized (PIC), and non-immunized (NIC) based on the national Expanded Program on Immunization schedule. Weighted district-level analyses were conducted to ensure representativeness and comparability. Between 2023 and 2025, immunization coverage improved across SHRUC districts. FIC increased from 62.4% to 73.3% (+10.9 percentage points), while PIC declined from 33.5% to 24.5% and NIC from 4.1% to 2.2%. The largest gains were observed in Balochistan, where FIC rose from 44.2% to 60.1% and NIC dropped from 12.3% to 3.8%. Sindh also showed strong progress, with FIC increasing from 60.4% to 73.6%. Khyber Pakhtunkhwa maintained high baseline coverage, with a modest increase from 81.3% to 83.0%. District-level variation persisted in 2025. Peshawar reported the highest coverage (83.0% FIC; 0.5% NIC), followed by Karachi West (77.4%), Karachi East (75.2%), and Quetta (74.4%). Karachi Malir (54.3%) and Pishin (57.3%) showed moderate performance but high partial immunization. Chaman remained lowest, with 38.6% FIC and 55.7% PIC. In conclusion, while coverage improved, inequities persist. High PIC levels highlight gaps in completion. Strengthening defaulter tracking, follow-up, and second-year-of-life vaccination is essential to convert PIC into FIC and sustain gains.

7016

ACUTE FEBRILE ILLNESS IN GEORGIA: INSIGHTS FROM PROSPECTIVE SURVEILLANCE

Magda Metreveli[1], Nora Kokaia[2], Manana Makharadze[2], Elene Pachkoria[3], Tamar Jajanidze[1], Shorena Mazmaniani[1], Levan Ratiani[3], Cody Phelps[1], Thomas Musich[1], Nino Trapaidze[1]

[1]Walter Reed Army Institute of Research-Europe and the Middle East, Tbilisi, Georgia, [2]Institute of Medical Parasitology and Tropical Medicine, Tbilisi, Georgia, [3]The First University Clinic of Tbilisi State Medical University, Tbilisi, Georgia

Acute febrile illness (AFI) remains a major challenge in Georgia, where limited diagnostics and overlapping symptoms hinder accurate identification of zoonotic and vector-borne causes. Service members operating in the country face a high risk of exposure to AFI pathogens. We conducted prospective hospital-based surveillance to characterize infectious causes of undifferentiated AFI. We enrolled patients aged ≥4 years who presented with fever ≥38°C for ≥48 hours without a confirmed diagnosis. We tested serum samples using an enzyme-linked immunosorbent assay (ELISA), serum agglutination test and immunoblotting to detect regionally relevant pathogens, including *Leishmania* spp., *Leptospira* spp., Crimean-Congo Hemorrhagic Fever Virus, *F. tularensis*, West Nile Virus, *R. typhi*, Spotted Fever, *Brucella* spp., *C. burnetii*, Tick-borne Encephalitis virus, *Borrelia* spp., and Hantavirus. A subset of samples underwent real-time PCR testing to

detect antigens. From April 2024 to March 2026, we enrolled 295 patients (mean age 48 years; range 12-80; 65% female). Serologic testing identified *Rickettsia typhi* as the predominant infection, with IgM antibodies detected in 85% of patients, followed by *Brucella* spp. (12%), *Leptospira* spp. (12%), *Borrelia* spp. (10%), Hantavirus (6%), and *F. tularensis* (5%). Overall, 48% of patients demonstrated IgM reactivity to ≥2 pathogens. In contrast, molecular testing did not identify any causative agents. These findings show that rickettsial and zoonotic infections are common, but overlapping serologic responses hinder identification of a single causative agent, emphasizing the need for integrated, region-specific diagnostic strategies to guide public health action. **Disclaimer:** Material has been reviewed by the Walter Reed Army Institute of Research. There is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the author, and are not to be construed as official, or as reflecting true views of the Department of the Army or the Department of Defense.

7017

RISK FACTORS OF SUSCEPTIBILITY TO MULTIPLE VACCINE-PREVENTABLE DISEASES AMONG CHILDREN IN NORTH DARFUR, SUDAN: INTEGRATED SEROSURVEILLANCE RESULTS FROM TRACHOMA IMPACT SURVEYS

Sarah Lapidus[1], Tania Gonzalez[2], Angelia Sanders[2], Balgesa E. Elshafie[3], Sara Lavinia Brair[4], Kelly Callahan[2], Amy Wesolowski[1], Sophie Berube[5], Sonia Hegde[1], Saki Takahashi[1], Scott Nash[2]
[1]Johns Hopkins University, Baltimore, MD, United States, [2]Atlanta, GA, United States, [3]National Program for Prevention of Blindness, Federal Ministry of Health, Khartoum, Sudan, [4]Carter Center, Khartoum, Sudan, [5]Gainesville, FL, United States

Vaccine-preventable diseases (VPDs) remain a leading cause of childhood morbidity and mortality in settings with suboptimal vaccination coverage. Integrated serosurveillance offers a valuable tool to identify VPD population immunity gaps, understand their drivers, and inform vaccination strategies. In 2019, population-based trachoma surveys in North Darfur, Sudan were conducted across 3 localities, and children provided a dried blood spot to test for antigens to trachoma and other diseases of public health importance. Here, we analyzed IgG serological responses to measles and tetanus (from the combined DTP vaccine) from 1,659 children ages 1-4 years. A multinomial logistic regression examined associations between seronegativity to measles and tetanus (i.e., "serological zero-dose status") and factors at the individual level (age, sex), household level (adult educational attainment, latrine access, livestock ownership), and cluster level (population size, travel time to health facility). Overall, seroprevalence was 65.3% for measles (95% CI: 63.0%–67.6%) and 75.0% for tetanus (95% CI: 72.9%–77.1%), with 13.6% of children seronegative to both antigens (95% CI: 12.1%–15.4%). Children in households with no educational attainment had higher odds of being seronegative to both antigens (OR: 3.01, 95% CI: 2.14–4.21), seropositive to measles only (OR: 1.93, 95% CI: 1.32–2.82), and seropositive to tetanus only (OR: 1.55, 95% CI: 1.12–2.16), relative to children seropositive to both antigens. Similarly, children in households without a functioning latrine had higher odds of being seronegative to both antigens (OR: 5.65, 95% CI: 3.51–9.10), seropositive to measles only (OR: 3.54, 95% CI: 2.32–5.40), and seropositive to tetanus only (OR: 1.45, 95% CI: 1.10–1.92). Overall, the absence of adult educational attainment and lack of latrine access was consistently associated with higher odds of seronegativity to measles and tetanus in children, with the strongest associations observed for dual seronegativity. Insights from integrated serosurveillance could be used to inform vaccination strategies to reduce VPD susceptibility.

7018

A RESEARCH INSTITUTION'S JOURNEY TO ISO ACCREDITATION (PROSPECTS AND CHALLENGES)

Eunice Obeng, Nancy-Maud Apiah, Susan Adu-Amankwah
Noguchi Memorial Institute for Medical Research, Accra, Ghana

Accreditation as a global imperative is increasingly gaining relevance for health systems in developing countries. While progressive strides have been made in clinical diagnostic laboratories for standards such as ISO 15189 for medical laboratories and ISO 17025 for testing laboratories, biomedical research laboratories are yet to catch up. Adapting to the quality paradigm presents opportunities which can be leveraged to enhance the continental picture of accredited biomedical research facilities across Africa. The Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana since September 2022 adapted a strategic approach to harmonize its quality operations. Management processes were centralized, roles more clearly defined, and performance monitoring indicators established. The institutional quality office was empowered to become more functional and expanded with quality coordinators assigned to promote compliance across all departments. Greater awareness was created to enhance the culture of quality throughout the institute. The outcomes are demonstrated in significant changes in behaviour and perception in favour of quality management systems in the institute, resulting in a successful ISO 15189:2022 accreditation. This opportunity further favours a holistic improvement of the entire organization. In conclusion, while accreditation opens avenues for collaboration, credibility, and enhanced quality, it is not without obstacles. Approximately 10 months of strategic implementation of quality management requirement exposed the need for development of a more specific international standard for biomedical research laboratories. Regardless of the challenges associated with QMS implementation its benefits far outweigh the challenges and must be required of all biomedical research facilities.

7019

A STANDARDIZED INCIDENCE-BASED METHODOLOGY FOR PRIORITIZING SUB-NATIONAL AREAS FOR PREVENTIVE ORAL CHOLERA VACCINATION

Zimy Wansaula[1], Lucy Breakwell Nagle[1], Molly Hancuh[2]
[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]CDC Foundation, Atlanta, GA, United States

Preventive oral cholera vaccination (OCV) is an important complementary strategy for cholera control in endemic settings. Given limited protection and global vaccine supply, systematic subnational prioritization is needed. CDC and partners developed an initial methodology incorporating epidemiologic indicators, vulnerability factors, and mortality, piloted in two countries in 2023. The pilots revealed key limitations - vulnerability factors were often incomplete or inconsistently measured, and mortality reflected health system gaps more than transmission risk - signaling the need to redesign the methodology to improve objectivity and better target endemic settings (defined as areas reporting cholera cases in ≥3 of the last 5 years). We conducted an iterative redesign informed by pilot feedback, expert consultations, and review of OCV planning experiences. Comparative analyses of epidemiological indicators supported adoption of an exclusively incidence-based framework. The revised methodology determines eligibility using minimum thresholds for annual incidence and years with reported cholera cases to capture areas with substantial burden and sustained transmission. Eligible areas are prioritized by mean annual incidence and variability and stratified into three tiers: (1) high incidence and stable transmission, (2) high incidence but variable transmission, and (3) lower incidence. Areas not meeting criteria remain candidates for reactive vaccination. We applied the revised method to cholera data used for planning preventive OCV campaigns in four countries. The tool reduced prioritized hotspots from 22-117 to 14-23, enabling more targeted vaccination. It also decreased requested OCV doses from 93.5 million to 59.3 million, reflecting more efficient prioritization. Continued piloting will further assess performance in settings with surveillance gaps

and heterogeneous epidemiology. The revised tool provides an objective approach for prioritizing preventive OCV use, helping direct limited supplies to areas where they can most effectively reduce long-term cholera burden.

7020

PLASMA PEPTIDOME SIGNATURES BY MALDI-TOF MS FOR SEPSIS DIAGNOSIS AND STRATIFICATION: A PILOT STUDY

Jessica Rocca[1], Facundo N. Urteaga[2], Mailén Di Palma[3], Monica Prieto[3], Nahuel Rubatto[4], Elisa Estenssoro[5], Pablo Schierloh[2], Bárbara Rearte[1]

[1]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET); Instituto Nacional de Enfermedades Infecciosas - ANLIS Malbrán, Ciudad Autónoma de Buenos Aires, Argentina, [2]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET); Instituto de Investigación y Desarrollo en Bioingeniería y Bioinformática (IIB)–CONICET UNER, Oro Verde - Entre Ríos, Argentina, [3]Instituto Nacional de Enfermedades Infecciosas - ANLIS Malbrán, Ciudad Autónoma de Buenos Aires, Argentina, [4]Sanatorio Otamendi y Miroli, Ciudad Autónoma de Buenos Aires, Argentina, [5]Escuela de Gobierno en Salud "Floreal Ferrara", Ministerio de Salud de la Provincia de Buenos Aires, La Plata, Provincia de Buenos Aires, Argentina

Sepsis is a major global health problem and a leading cause of morbidity and mortality in intensive care units (ICU). Early diagnosis and patient stratification remain challenging due to the heterogeneous nature of sepsis and the limited sensitivity of blood cultures. Proteomic approaches combined with artificial intelligence represent a promising strategy in biomedical research. We previously showed that plasma peptidome analysis by MALDI-TOF MS discriminates pathophysiological stages in experimental models and critically ill COVID-19 patients. This study evaluated the potential of plasma peptidome signatures for sepsis diagnosis and stratification. In this pilot prospective cohort study, 18 ICU patients were classified as septic (S, n=10) or non-septic (NS, n=8). Clinical data and serial peripheral blood samples (days 1, 3, 5, 7) were collected for plasma peptidome analysis by MALDI-TOF MS and immunophenotyping by flow cytometry (days 1 and 7). Healthy subjects (H, n=8) were included as controls. A total of 576 spectra were analyzed using unsupervised clustering (PAM) and supervised approaches (Random Forest and Logistic Regression) to assess classification performance. No significant differences were observed between S and NS by flow cytometry. Compared with H, S patients showed reduced T and B lymphocytes (% median [IQR]: T $CD4^+$ S, 24.4 [13.2-31.2] vs H, 39.5 [26.2-51.9], $p < 0.05$; B $CD19^+$ S, 10.5 [5.2-14.7] vs H, 21.4 [14.7-28.9] $p < 0.05$), increased immature neutrophils ($CD11b^+/CD10^-$: S, 30.2 [6.3-40.4] vs H, 2.6 [1.1-4.4] $p < 0.05$) and expanded PD-$L1^+$ intermediate monocytes ($CD14^+$/PD-$L1^+$: S, 46.0 [34.4-74.6] vs H, 3.9 [1.3-4.5] $p < 0.05$), consistent with an immunosuppressive phenotype. In plasma peptidome analysis, machine learning models discriminated S vs NS patients with high performance (Random Forest: accuracy 86%, sensitivity 88%, specificity 82%, AUC 0.88). Despite limited discrimination by clinical and immunological variables in S vs NS patients, plasma peptidome analysis enabled accurate classification, supporting the potential of plasma peptidome signatures for early diagnosis and stratification in sepsis.

7021

DEVELOPING A POINT OF CARE ULTRASOUND TRAINING PROGRAM IN RESOURCELIMITED ACADEMIC SETTINGS: A BLENDEDLEARNING APPROACH FROM RURAL NEPAL

Juan Lopez Tiboni

Pennsylvania Hospital of the University of Pennsylvania, Philadelphia, PA, United States

Point of care ultrasound (POCUS) has risen in prominence due to its practical utility and accessibility as a clinician-implemented imaging modality, with the increasing low-cost and portability of ultrasound giving it a scalability that exceeds that of more physically cumbersome and expensive imaging domains. In recent years POCUS has become of increasing interest within the humanitarian and capacity building space in low and middle income countries, where many elements of clinical medicine are practiced in the absence of advanced imaging technologies. Low- resource health settings often prioritize immediate patient care needs over the adoption of new technologies or educational innovation, and innovative tools can come from humanitarian initiatives directly at the point of care through field hospitals or NGO managed healthcare. The best way to maximize possible benefit from POCUS is to focus on programs that introduce POCUS as part of a healthcare education, whether that be at medical student or residency level. This paper describes a blended learning approach to POCUS training developed in rural Nepal, combining online didactic content with intensive in-person practical training. The program featured a five-day bootcamp covering fundamental ultrasound physics, FAST examination, renal ultrasound, cardiac imaging, lung ultrasound, DVT examination, procedural guidance, and pathology cases, supplemented by hands-on practice sessions. The curriculum was designed to be integrated into existing medical education structures, addressing the unique challenges of introducing novel educational elements into low and middle income country healthcare training programs. This model demonstrates a sustainable approach to building POCUS capacity in resource-limited academic settings through partnerships that work within existing educational frameworks.

7022

READINESS OF COMMUNITY HEALTH WORKERS TO DELIVER INTEGRATED COMMUNITY CASE MANAGEMENT IN NIGERIA: FINDINGS FROM THE ROTARY HEALTHY COMMUNITIES CHALLENGE BASELINE ASSESSMENT

Ekpeme Obi[1], Gloria Sillo[1], Chinedu Egwuonwu[1], Meredith Center[2], Todd Jennings[3], Ayebatari Lawson[1], Dare Eguavon[1], Murtala Muhammad[1], Eno Angela Attah[4], Abubakar Muhammad Noma[5]

[1]PATH, Abuja, Nigeria, [2]PATH, Bozeman, MT, United States, [3]PATH, Lusaka, Zambia, [4]Akwa Ibom State Primary Health Care Development Agency, Akwa Ibom, Nigeria, [5]Kebbi State Primary Health Care Development Agency, Kebbi, Nigeria

Integrated community case management (iCCM) in Nigeria is delivered through volunteer Community Health Workers (CHWs), yet limited evidence exists on their readiness for iCCM service delivery. The Rotary Healthy Communities Challenge (RHCC) project conducted a cross-sectional baseline assessment in project sites across 12 LGAs in Akwa Ibom and Kebbi States of 270 CHWs and 421 caregivers, complemented by a desk review of state systems. Although all CHWs had received initial training, they identified malaria, diarrhea, pneumonia management, and ANC promotion as priority training needs. Most CHWs resided within their assigned communities (88% Akwa Ibom; 77% Kebbi) and delivered services an average of three days per week. Among CHWs residing outside their community, those in Kebbi frequently (46%) reported travel times exceeding one hour, thereby constraining service availability. Nearly all agents (99%) believed their communities were aware of their services, yet only 21% in Akwa Ibom and 46% in Kebbi felt they were the first choice for malaria testing or treatment. Recent inactivity affected 5% of CHWs in Akwa Ibom and 14% in Kebbi, due to stockouts of essential medicines, irregular supervision, and inconsistent payment of stipend. Desk review findings related to CHWs revealed no routine monthly reporting, poor documentation, or stockout of iCCM commodities. Caregivers' knowledge of iCCM and available services was 72%, with higher awareness in Kebbi (81%) than in Akwa Ibom (59%), and most (96%) caregivers are likely to seek iCCM services for their child from CHWs. Key barriers to timely care-seeking included limited recognition by the caregivers of pneumonia symptoms and danger signs, and distance to facilities. The RHCC project is working with the Governments of Akwa Ibom and Kebbi States to address identified gaps by strengthening lastmile commodity delivery, institutionalizing supportive supervision, establishing effective data reporting and information management processes, to improve the functionality of CHWs to deliver iCCM in Nigeria.

7023

ESTABLISHING BEST PRACTICES FOR INVENTORY MANAGEMENT SYSTEMS FOR HEALTH COMMODITIES: THE CASE OF BIOKO ISLAND

Tony Kazungu[1], David Amador[1], Raul Memba Boriba[1], Miriam Riobi Patatobe[1], Matilde Riloha Rivas[2], Wonder Philip Phiri[1], David Galick[1], Carlos Alberto Guerra[3], Guillermo A. Garcia[4]

[1]MCD Global Health, Malabo, Equatorial Guinea, [2]National Malaria Control Program, Malabo, Equatorial Guinea, [3]MCD Global Health, Quito, Ecuador, [4]MCD Global Health, Hallowell, ME, United States

Managing health commodity supply across health facility networks requires real-time visibility into stock levels, consumption patterns, and distribution schedules. Many programs rely on fragmented data sources and manual processes that limit this visibility and slow responses to stockouts and supply gaps. The Bioko Island Malaria Elimination Project (BIMEP) manages commodity supply across 31 public health facilities on Bioko Island through a direct distribution model, and across mainland Equatorial Guinea through a regional warehouse and district-level supply chain. Commodities include malaria drugs, diagnostics, cervical cancer supplies, and other health program materials. Drugs are prepacked into patient-level treatment packages in a dedicated warehouse before distribution to health facilities. In 2026, BIMEP implemented an integrated inventory management system combining ARBIMed as the core stock management platform, DHIS2 for service delivery and consumption tracking, Python-based ETL pipelines to connect data sources, Power BI dashboards for real-time visualization, and Microsoft Lists to coordinate distribution scheduling and routine facility visits. Standardized protocols govern stockroom operations across all sites. In the first six months of operation, the system tracked six distribution cycles across both warehouses and identified six stockouts, each of which triggered a direct resupply response from the BIMEP logistics team. Real-time stock visibility across facilities and warehouses is now available for the first time, enabling proactive rather than reactive supply chain management. Integrating commodity, service delivery, and operational data into a single system with automated dashboards and standardized workflows is a practical and replicable model for health commodity management in any setting where supply is managed across multiple facilities and supply chain tiers.

7024

PULMONARY FUNCTION AND RESPIRATORY SYMPTOMS AMONG A COHORT OF TEA FACTORY AND ESTATE WORKERS IN A SRI LANKAN TEA PLANTATION

Vinura Kuruneru, Thashini Nethma, Jayani Hennayake, **Hermali Silva**

University of Colombo, Colombo, Sri Lanka

Tea is one of the main exports of Sri Lanka and it plays a significant role in the economic growth of the country. Is it equally important that the employees involved in the tea manufacturing process are of optimal health as it directly affects their efficiency and impact on the tea industry as well as the economy as a whole. With tea dust being one of the major potential health hazards, this study was done to compare respiratory symptoms and pulmonary function between the workers in the shifting area (tea dust-exposed) and estate (non-exposed) workers. This cross-sectional study involved 240 workers: 120 sifting area and 120 tea estate workers. Data on socio-demographics, respiratory symptoms, and pulmonary function (PEFR) were collected using validated questionnaires, peak flow meters, and anthropometric measurements. Ethical clearance, informed consent, and quality control were ensured, and data were analysed with descriptive and inferential statistics. Sifting area workers were mainly female (85%), married (95%), Sinhalese (98%), and educated up to O/Ls (54%), while tea estate workers were 53% female, 76% married, 70% Sinhalese, with 51% having no formal schooling. Mean age was higher in sifting area workers (49.1 vs. 42.0 years), and service duration was longer in tea estate workers (11.8 vs. 5.7 years). Daily working hours were similar; mean BMI was slightly higher in sifting area workers than in the estate area (20.4 vs. 19.2 kg/m^2). Respiratory symptoms were more prevalent among sifting area workers than among the estate workers: cough (35.8% vs. 22.5%), chronic cough (14.2% vs. 3.3%), and chronic phlegm (26.7% vs. 15.8%) were significant. Prevalence of breathlessness was higher among sifting area workers (n=48, 40%) than tea estate workers (n=36, 30%). The most prevalent respiratory symptom among both sifting area and estate workers was phlegm which was more prevalent in sifting area workers (n=67, 55.83%). PEFR was lower in sifting area workers than in estate workers (363.8 vs. 420.1 L/min, p=0.001). Findings highlight occupational risks from tea dust exposure, emphasizing the need for safety measures , awareness and further research.

7025

ACCELERATING GLOBAL HEALTH PROTECTION: THE HUMANIZED HIS-DRAGA MOUSE AS A PRECLINICAL PLATFORM FOR TROPICAL INFECTIOUS DISEASE COUNTERMEASURES

Sofia Casares[1], Sounak Ghosh Roy[2], Ahmad Faisal Karim[2], Liliana K. Thron[3], Jiae Kim[4], Pamela J. Skinner[3], Teodor-D Brumeanu[5], Mangala Rao[6]

[1]Naval Medical Research Command, Silver Spring, MD, United States, [2]Henry M Jackson Foundation for the Advancement of Military Medicine, Silver Spring, MD, United States, [3]University of Minnesota, Minneapolis, MN, United States, [4]MHRP at the Walter Reed Army Institute of Research (WRAIR), Silver Spring, MD, United States, [5]Uniformed Services University, Bethesda, MD, United States, [6]Silver Spring, MD, United States

Developing highly effective medical countermeasures against diverse tropical and emerging infectious diseases is a major global public health priority. Historically, standard mouse models do not accurately mimic human immune responses, which impedes translational research for complex pathogens. To bridge this gap, the Human Immune System (HIS)-reconstituted DRAGA (HLA-A2.HLA-DR4. Rag1KO.IL2RγcKO.NOD) mouse serves as a highly predictive surrogate *in vivo* human model. Reconstituted with a functional human immune system from HLA-compatible cord blood stem cells, these mice enable rigorous preclinical tests of vaccines and drugs against severe tropical and global health threats. These include malaria (*Plasmodium falciparum*), scrub typhus (Orientia tsutsugamushi), dengue, Zika virus, Hantaan viruses, HIV, influenza, and SARS-CoV-2. The HIS-DRAGA model advances research into chronic infections by supporting persistent viral replication, modeling latency, and enabling the evaluation of antiretroviral therapies and next-generation cellular immunotherapies, such as CAR-T and CAR-NK cells. Crucially, the discovery of functional human microglia and resident T cells within the HIS-DRAGA brain provides a unique model to study neuroinflammation and central nervous system targeting tropical pathogens, which often complicate vector-borne diseases. Furthermore, this model serves as an agile platform to create fully human monoclonal antibodies (hu-mAbs) against emerging tropical threats. By isolating human B cells from immunized DRAGA mice, we have successfully generated targeted hu-mAbs against *P. falciparum,* influenza, SARS-CoV-2, hantavirus, CCHF, and SFTS. These are currently being evaluated for cross-strain reactivity and therapeutic efficacy. By bridging the translational gap between animal models and human clinical trials, the HIS-DRAGA platform speeds up the global medical countermeasure development pipeline. This ensures enhanced pandemic preparedness and bolsters global health security against the most pressing tropical infectious diseases affecting vulnerable populations all around the world.

7026

RISK FACTORS AND CLINICAL PRESENTATION ASSOCIATED WITH ABNORMAL CERVICAL CANCER SCREENING OUTCOMES IN EQUATORIAL GUINEA: INSIGHTS FROM A LARGE CERVICAL CANCER SCREENING PROGRAM

Liyu A. Teklemichael[1], Carolina Amadou Muana[2], José Avelino Rondo Massoko[2], María Jesús Siribola Bolecha[2], Manuel Ondo Oyono[2], Wonder Philip Phiri[2], Beulah Jayakumar[3], Guillermo A. Garcia[1], Jordan Smith[1], Carlos Alberto Guerra[4], Antonio Enrique Ngua Sama Roca[2]

[1]MCD Global Health, Hallowell, ME, United States, [2]MCD Global Health, Malabo, Equatorial Guinea, [3]MCD Global Health, Delhi, India, [4]MCD Global Health, Quito, Ecuador

A cervical cancer screening and treatment program has operated in Equatorial Guinea since 2017. Women between 20 and 60 years old receive free screening using visual inspection with acetic acid (VIA) and Lugol's iodine (VILI), and treatment if positive. During screening, data on demographics, risk factors (namely, smoking, parity, and age of first sexual intercourse (AFSI)), and clinical presentation are collected. We analyzed these data to identify factors related to VIA/VILI positivity. By March 2026, 29,770 eligible women had been screened, with 5,535 (18.6%) with symptoms, including, post-coital bleeding (PCB; 230/5,535, 4.2%), dyspareunia (528/5,535, 9.5%), vaginal discharge (4,055/5,535, 73.3%), genital itching (545/5,535, 9.8%), ulcers (74/5,535, 1.3%), and lower abdominal pain (2,814/5,535, 50.8%). Smoking was reported by 382/29,770 (1.3%). AFSI ranged between 10 and 48 years. Of the total screened, 692 (2.3%) tested positive; of these, 420/24,235 (1.7%) were asymptomatic, 61/230 (26.5%) presented with PCB, and 46/528 (8.7%) with dyspareunia. PCB and dyspareunia were most strongly associated with a positive result in univariate regression; a composite symptom burden (CSB) variable added all other reported symptoms together. Multivariate logistic regression included PCB, dyspareunia, CSB, age, AFSI, smoking, and parity as covariates, and positive VIA/VILI as the response. After adjusting, PCB was the factor most strongly associated with an abnormal outcome (aOR: 7.97; 95% CI: 5.54-11.42; $p < 0.001$), followed by dyspareunia (aOR: 2.13; 95%CI:1.48-2.98; $p<0.001$), and smoking (aOR: 1.89; 95% CI: 1.07-3.13; $p = 0.05$). CSB, AFSI or parity were not significantly associated. Age was inversely related to positivity, each additional year corresponding to a modest reduction in the odds (aOR: 0.98; 95% CI: 0.97-0.99; $p < 0.001$). While the findings from this large data set confirm existing evidence of the clinical presentation of cervical cancer and its risk factors, their implications for cervical cancer screening in general, and in the context of primary health care in particular, will be discussed.

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PAEDIATRIC PAIN MANAGEMENT PRACTICES IN NORTHERN GHANA: AN OBSERVATIONAL STUDY

Francisca Achaliwie[1], Margaret W. Kukeba[2], Jamilatu B. Kappiah[2]

[1]Department of General and Preventive Health Nursing, School of Nursing and Midwifery, University of Technology and Applied Sciences, Navrongo, Ghana, [2]Department of Maternal and Child Health Nursing, School of Nursing and Midwifery, University of Technology and Applied Sciences, Navrongo, Ghana

Pain is very common in hospitalised children, which is already known to be undertreated. Inadequate management of pain in children can result in a number of detrimental patient outcomes. However, effective paediatric pain management practices by nurses in developing countries, including Ghana, are underexplored. This study therefore aimed to explore the paediatric pain management practices by nurses in a municipal hospital in northern Ghana. A qualitative design was employed to conduct the study. Data collection was undertaken via observation between November 2023 and April 2024. Fifteen (15) nurses were shadowed on all shifts. A total of 114 hours of observations were undertaken. Participants included nurse assistants, registered general nurses and paediatric nurse specialists. Data analysis drew on a thematic analysis framework. Findings revealed inadequate paediatric pain management practices. No nurse was observed assessing pain. Acetaminophen was observed as the commonly administered pain medication, while the only non-pharmacological intervention was distraction. Additionally, several children were observed undergoing painful procedures such as cannulation and intravenous draws without any pain management intervention. Communication between nurses and children/parents about pain was almost non-existent. The nurses had undertaken a workshop on paediatric pain management in December 2023. However, their pain management practices remained largely the same afterwards. Paediatric pain management practices were woefully inadequate in the children's ward; this could be attributed to lack of policy guidelines for pain management. There is, therefore, the need for guidelines that would become a routine part of clinicians' work to enhance the assessment and management of pain.

7028

COMMUNITY-DRIVEN VACCINATION - AN INNOVATIVE, COMMUNITY-BASED APPROACH TO REDUCING ZERO-DOSE AND UNDER-VACCINATED CHILDREN IN MADAGASCAR

Jerry Rakotozafy[1], Liyu Teklemichael[2], Jordan Smith[2], Duncan McFarland[2], Josea Ratsirarson[2], Ellen Knowles[2], Guillermo Alberto Garcia[2], Carlos Alberto Guerra[3], Rija Fanomeza[1]

[1]MCD Global Health, Antananarivo, Madagascar, [2]MCD Global Health, Hallowell, ME, United States, [3]MCD Global Health, Quito, Ecuador

Community-driven vaccination (CDV) to accelerate immunization of zero-dose or under-vaccinated (ZDUV) children is an innovative, community triggering method inspired by the community-led total sanitation approach, which aims to foster a sustainable dynamic of vaccine demand, child follow-up, and collective accountability around children's health, leading community stakeholders to recognize the risks associated with non-vaccination and to decide on corrective actions. Implementation follows four complementary steps: pre-triggering, institutional triggering, community triggering, and follow-up. The approach was launched in September 2025 across 12 districts and 29 communes in Madagascar and mobilizes local authorities and health center managers, community health workers, traditional leaders, households, and other local influencers to identify ZDUV children, analyze barriers, and agree on concrete solutions. The project aims to ultimately vaccinate 35,000 children from priority communities. A baseline household survey in June 2025, which included 301 children between 6 and 23 months old, revealed that only 33.2% had complete immunization coverage and 5% had not received any vaccine. Continuous community generated data are collected primarily by community agents and monthly digitized by technical assistants. Results from this routine data collection show that between September 2025 and February 2026 15,309 mothers had been sensitized about the importance of vaccinating their children, and that 12,802 ZDUV children had been vaccinated (36.5% of those targeted for immunization). Despite several challenges faced during implementation, these preliminary results suggest that the approach promotes rapid community mobilization in favor of vaccination to reduce the number of ZDUV children. At a larger scale, CDV should enable better resource targeting, improved coordination between communities and health services, and stronger accountability around vaccination outcomes. The approach offers a credible foundation for a sustainable and replicable model for reaching ZDUV children.

7029

A SCALABLE DATA ANALYSIS PIPELINE FOR MULTIPLEX BEAD ASSAY SEROSURVEILLANCE STUDIES

Viswanathan V. Krishnan, Ph.D.[1], Nishanth Parameswaran[1], Kumar V. Udhayakumar[2], Neeraja Venkateswaran[3], Kodumudi Venkateswaran[1]

[1]Tetracore Inc., Rockville, MD, United States, [2]Task Force for Global Health, Decatur, GA, United States, [3]Tetracore Inc, Rockville, MD, United States

Multiplex bead assay (MBA) platforms facilitate high-throughput, cost-effective measurement of antibody responses to multiple antigens simultaneously, and their use is increasingly prevalent in large-scale serosurveillance. Data generated across multiple plates and study sites necessitate standardized methods for preprocessing, quality control (QC), and reactivity calling to ensure reproducibility and comparability. We have developed an integrated data analysis pipeline supporting both site-specific single-plate review and harmonized multi-plate, multi-site analysis for serosurveillance studies. A custom 16-Plex FlexImmArray (FIA) arbovirus multiplex assay was used for a multi-center evaluation and validation of the data analysis pipeline. We developed the Tetracore® ShinyFIA App, combining open-source R tools for reading, QC, and preprocessing MBA data with interactive Shiny-based applications for visual review and reactivity assessment. For single-plate analysis, raw xPONENT® / Bioplex Manager® outputs are evaluated for plate layout integrity, median fluorescence intensity distributions, and reference control performance. Bead-specific cutoffs are estimated using an external calibrator and QC samples, and preliminary reactivity calls are generated through the Tetracore ShinyFIA workflow, with site-level outputs including QC summaries and preliminary reports to support rapid review and feedback. For multi-plate or multi-site analysis, plate-level outputs are harmonized into structured objects to facilitate cross-plate comparisons, consistency assessment, and study-wide reporting. Automated workflow in the App standardizes control assessment, bead-level cutoff estimation, reactivity classification, and plate-to-plate QC review. Testing across plates demonstrated consistent assay performance, confirming the robustness of the approach for larger applications. This scalable pipeline provides a practical framework for standardized MBA preprocessing, QC, and integrated downstream analysis, with the potential to enhance reproducibility and comparability across laboratories.

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PREVALENCE AND DETERMINANTS OF DEATH REGISTRATION ACROSS THREE DEMOGRAPHIC SURVEILLANCE SITES IN KENYA

Caroline B. Osoro[1], James Bukosia[1], Isaac Waluke[1], Peter Mwangala Sifuna[2], Kenedy Omondi Omolo[2], Amek Nyaguara[3], Vincent Were[4], Mike Rajwayi[1], Barack Mwanzia[1], Sharon A. Onyango[1], Viola Chepkurui[1], Christopher Nyundo[3], Joseph Kamau[5], Stephané Helleringer[6], Robert Snow[7], Emelda Okiro[7]

[1]KEMRI-Wellcome Trust Research Programme, Nairobi, Kenya, [2]KEMRI-Walter Reed Army Institute of Research, Kisumu, Kenya, [3]KEMRI-Wellcome Trust Research Programme, Kilifi, Kenya, [4]African Population Health Research Centre, Nairobi, Kenya, [5]Department of Civil Registration Services, Nairobi, Kenya, [6]New York University, Abu Dhabi, United Arab Emirates, [7]University of Oxford, Oxford, United Kingdom

Continuous recording of vital events is essential for evidence-based planning in healthcare. However, Africa and Asia have the lowest reported rates in death registration, as evidenced during the COVID-19 pandemic. In Kenya, national death registration declined from 52.3% in 2021 to 44.8% in 2024. We assessed the completeness of death registration and its determinants across three Health and Demographic Surveillance System (HDSS) sites in Kenya. We conducted household surveys, sampling 1,363 households with a recent death. We quantified death registration completeness, evaluated determinants of death registration using random-effects regression analysis, and characterised reasons for registration and non-registration. Among 1,387 deaths, only 257 (18.5%) were reported as registered, with similarly low completeness in both rural and urban settings. Registration was lowest for deaths among infants (OR 0.09; 95% CI 0.03-0.26; $p < 0.001$) and children 1-4 years (OR 0.14; 95% CI 0.04-0.48; $p = 0.002$) compared with adults 65-84 years. It was lower among the deceased who were unmarried (OR 0.38; 95% CI 0.17-0.84; $p = 0.016$), unemployed (OR 0.60; 95% CI 0.40-0.89; $p = 0.011$), and those who died at home (OR 0.65; 95% CI 0.47-0.90; $p = 0.010$). Deaths in urban settings were more likely to be registered (OR 1.55; 95% CI 1.07-2.24; $p = 0.020$). There were higher odds of registration in households with greater wealth (OR 2.50; 95% CI 1.52-4.11; $p < 0.001$), and among deceased who were employed or retired (OR 1.87; 95% CI 1.27-2.74; $p = 0.001$). Recent deaths (0-6 months) were least likely to be registered: (OR 0.26; 95% CI 0.14-0.48; $p = <0.001$). Lack of awareness, high costs and perceived lack of benefits were the leading reasons for non-registration. Death registration in Kenya is low, delayed and influenced by the deceased's socio-demographic characteristics, setting and place of death. Information and advocacy campaigns on the importance of timely death registration, particularly of children's deaths, are called for with targeted emphasis on the poor and unemployed. This study is important because there is a paucity of similar research, particularly in Africa.

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EVALUATING RISK COMMUNICATION AND COMMUNITY ENGAGEMENT TO SUPPORT EBOLA VACCINE UPTAKE DURING THE 2025 KASAÏ OUTBREAK, DEMOCRATIC REPUBLIC OF THE CONGO: A QUALITATIVE ASSESSMENT

Trésor Zola Matuvanga[1], Enock Nzembela[1], Gustave Lutumba[1], Albert Muya Dibaya[2], Hypolite Muhindo-Mavoko[1], Japhet Kabalu Tshiongo[1], Pierre Muhoza[3], Shibani Kulkarni[3], Terri Hyde[3], Emma Aberle Grasse[3], Didine Kaba[4], Jules Bukasa Kabongo[5], Pierrette Mubadi Ngankey[6], Patrick Mulopo Mitashi[1], Kimberly E. Bonner[3]

[1]Clinical Research and Immunization, Department of tropical Medicine, University of Kinshasa, Kinshasa, Democratic Republic of the Congo, [2]Service of Knowledge Management, SANRU ASBL, Kinshasa, Democratic Republic of the Congo, [3]Global Immunization Division, Centers for Disease Prevention and Control, Atlanta, GA, United States, [4]Department of Epidemiology and Biostatistics, School of Public Health, Kinshasa, Republic of the Congo, [5]Expended Programme on Immunisation, Ministry of Health, Hygiene and Prevention, Kinshasa, Democratic Republic of the Congo, [6]Centre des Opérations d'Urgence de Santé Publique (COUSP), Ministry of Health, Kinshasa, Democratic Republic of the Congo

The Democratic Republic of the Congo declared its 16th Ebola outbreak in Kasaï Province on September 4, 2025. Vaccination with the rVSVΔG-ZEBOV-GP (Ervebo®) Ebola vaccine began nine days later, with 47,577 doses administered over two months. Risk communication and community engagement (RCCE) activities were implemented to increase awareness, address concerns, and promote vaccine uptake in the community. This study evaluated how RCCE activities and coordination efforts influenced community acceptance and identified barriers and facilitators to vaccination among healthcare workers (HCWs), frontline workers (FLWs), community leaders, and community members.Focus group discussions were conducted with community members (8 groups), HCWs (8 groups), and FLWs (4 groups) in purposively selected vaccination-eligible health areas. Key informant interviews were conducted with community leaders and vaccination coordinators (n=17). Audio-recorded discussions were transcribed and analyzed using combined inductive and deductive thematic coding. High perceived risk of Ebola and its fatality rate were primary facilitators of vaccine acceptance. RCCE activities including community mobilization, radio messaging, and engagement with religious leaders and traditional healers increased vaccine awareness and trust in vaccination. Vaccination of HCWs reinforced confidence by demonstrating vaccine safety. Overall vaccine confidence and uptake were perceived as high; however, concerns regarding vaccination during pregnancy were reported and then addressed through ongoing RCCE efforts.The rapid deployment of Ebola vaccination following outbreak declaration, coupled with coordinated RCCE efforts may have contributed to vaccine acceptance during the Kasaï outbreak. Overall, trusted communication channels for RCCE activities and visible vaccination of HCWs contributed to vaccine confidence, despite

some initial concerns regarding vaccine safety during pregnancy. These findings can inform outbreak preparedness and strengthen RCCE strategies for Ebola vaccine deployment.

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ENTOMOLOGICAL SURVEILLANCE AND MOLECULAR DETECTION OF *ORIENTIA TSUTSUGAMUSHI* AND *LEPTOSPIRA* SPP. AMONG RODENTS AND CHIGGERS IN CAUVERY DELTA REGION, TAMIL NADU, INDIA

Sathya Jeevitha Balakrishnan[1], Antony Selvakumar[2], Govindarajan Renu[3], Vigil Anbiah[4], P K Srivastava[5], Rakesh Mishra[2], Mansi Rajendra Malik[2], Jayalakshmi Krishnan[1]

[1]Central University of Tamil Nadu, Tamil Nadu, India, [2]Tata Institute for Genetics and Society, Bangalore, India, [3]ICMR - Vector Control Research Centre, Tamil Nadu, India, [4]Annamalai University, Chidambaram, India, [5]Ex. National Vector Borne Disease Control Programme, Delhi, India

Scrub typhus and its global public health threat posed by synanthropic rodent-associated zoonoses reinforce its public health priority and integrated surveillance for this neglected rickettsial disease. This study on integrated vector and molecular surveillance of zoonotic pathogens within rodent hosts and chiggers was conducted in Thiruvarur district, due to absence of such evidence. Eco-epidemiological study through landscape-stratified sampling approach was undertaken in five distinct ecological settings. Systematically, 282 small-mammal species were trapped, morphologically identified, and confirmed using COI barcoding. The collected ectoparasites were identified taxonomically, and tissue samples of rodents and chiggers were screened for pathogens using duplex real-time PCR. The predominant host species included *Rattus rattus*, *Bandicota indica*, *Suncus murinus*, and *Milardia meltada* harboring ectoparasites of high zoonotic importance of *Leptotrombidium deliense*, *Leptotrombidium rajasthanensis*, *Schoengastiella ligula*, *Trombicula hypodermata*, *Microtrombicula spp*., *Xenopsylla cheopis*, and *Haemaphysalis spp.* Overall ectoparasite prevalence was 47.51%, with statistically significant landscape-dependent variations in host-ectoparasite associations ($\chi^2 = 12.92$, $p < 0.05$). Real-time duplex PCR results revealed *Orientia tsutsugamushi* with highest detection in hepatic (63.6%) and brain (57.4%) tissues, with progressively lower detection in renal (39.3%), lung (33.3%), and splenic (18.8%) tissues. *Leptospira spp*. were detected in 56.3% to 62.1% of examined tissues. Of the 347 chiggers analyzed, *O. tsutsugamushi* was detected in 42.4% and *Leptospira spp*. in 21.3%, and co-infection in 17.0%, indicating substantial enzootic co-circulation. This study provides the first entomological and molecular baseline for scrub typhus transmission in the Cauvery delta region. The observations highlight the call for evidence-based integrated vector control and One Health-based surveillance to clarify zoonotic transmission and strengthen public health response.

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LIMITED DURABILITY OF IMPROVEMENTS IN INFECTION PREVENTION AND CONTROL PRACTICES FOLLOWING REACTIVE INTERVENTIONS LEAVES HEALTHCARE FACILITIES VULNERABLE TO EBOLA VIRUS TRANSMISSION

Joy Yang[1], Kasereka Masumbuko Claude[2], Emily Kimani[3], Michael Hawkes[1]

[1]University of British Columbia, Vancouver, BC, Canada, [2]Université Catholique du Graben, Democratic Republic of the Congo, [3]University of Alberta, Edmonton, AB, Canada

The recent 2025 Ebolavirus disease outbreak (EVD) in the Democratic Republic of Congo, the sixteenth recorded, included healthcare worker deaths, inviting exploration of infection prevention and control (IPC) effectiveness in preventing nosocomial spread. We evaluated the outcomes and durability of an IPC bundle in ninety-two healthcare facilities in the DRC during the 2018-2020 Kivu EVD outbreak. IPC compliance was assessed using a 36-item standardized scorecard at baseline, immediately post-intervention, and at six-month follow-up. Baseline median IPC score was 19 (IQR 14-26). Following IPC bundle implementation, the median IPC score increased to 30 (IQR 28-33; $p<0.0001$), with improvement in 29 of 36 scorecard items (81%). Indicators with high baseline compliance, including waste incineration practice, Ebola alert systems, and response team activation protocol, showed no immediate improvement. At six-months follow-up, gains in IPC scores deteriorated to 28 (IQR 24-32; $p<0.0001$). Reductions in compliance were observed in 7 of 36 items (19%), notably in PPE availability, PPE wear by cleaning and waste-handling staff, triage and screening procedures, and on-site supervision. In conclusion, Despite extensive national experience with EVD outbreaks, IPC capacity in Congolese healthcare facilities remain vulnerable to erosion without sustained investment. Our study highlights the need for durable IPC strengthening to prevent healthcare-associated spread and protect healthcare workers in future EVD epidemics.

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WHAT WOULD POST-DISEASE-CONTROL SURVEILLANCE FOR SOIL-TRANSMITTED HELMINTHS REQUIRE? REVIEW OF TECHNICAL CHALLENGES, HEALTH SYSTEM BARRIERS AND ENABLERS, AND LESSONS FROM ANALOGOUS DISEASE PROGRAMMES

Mark Minnery[1], Kate Elizabeth McCracken[2], Tope Ogunbi[1], Ima Chima[1], Tanaya Raj[3]

[1]Evidence Action, Washington DC, DC, United States, [2]Durham, NC, United States, [3]Washington, DC, United States

Mass drug administration (MDA) for soil-transmitted helminths (STH) has brought some countries to less than 2% prevalence among school aged children, where WHO recommends transitioning to event-based preventive chemotherapy. To mitigate risk of losing gains made after years of MDA, an early warning surveillance system is necessary. Because post-MDA infections are overwhelmingly light-intensity, asymptomatic, and increase in intensity with repeated reservoir exposure, passive surveillance is unlikely to detect resurgence before morbidity re-establishes at population level. This suggests that active surveillance; small, periodic, quality-assured surveys in high-risk areas for signal detection, may be an optimal monitoring modality. We conduct a narrative review examining what an effective active STH surveillance system requires, synthesizing technical, health system, decision-making, and comparative evidence. Despite various geographies below 2% prevalence, we found no operational post-disease-surveillance system. Technically, diagnostic sensitivity at low intensity, sample timing requirements, and parasite identification assurance are primary constraints, though AI-assisted slide reading may offer partial mitigation. At the health system level, governance gaps, collapsing external financing, laboratory competency degradation, and fragmented data infrastructure are substantial barriers, partially offset by historical survey data, trained field and laboratory networks, geostatistical targeting tools, and existing NTD governance structures that may anchor surveillance without new institutional investment. On decision-making, prevalence-morbidity relationship supports a graduated response framework scaling monitoring frequency, survey scope, and treatment reinstatement to resurgence strength. Analogous disease programs offer transferable governance principles and potential integration. Global progress on STH disease control creates both urgency and opportunity to establish; evidence base, empirical thresholds, and health system frameworks, for successful surveillance.

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TIME SERIES ANALYSIS OF REPORTABLE DISEASE IN CHAD, 2014-2023

Margaret Louise Anderson[1], Sophie Licostie[2], Abdallah D. Adoum[3], Ali M. Moussa[4], Sten H. Vermund[5]

[1]Yale School of Public Health, Washington, DC, United States, [2]Yale School of Public Health, New Haven, CT, United States, [3]Ministry of Public Health, N'Djamena, Chad, [4]University of N'Djamena, N'Djamena, Chad, [5]University of South Florida, Tampa, FL, United States

Retrospective trend analyses of disease surveillance describe historical trends drive data informed decision-making and improve epidemic and

pandemic control. Using ten-year trends in surveillance data from Chad's National Surveillance System, we sought to characterize current disease trends to inform outbreak detection and response. National surveillance data were centralized in R, excluding incomplete disease categories and aggregating case notifications across six diseases for 2014-2023. We employed national case count time series, incidence heat maps, and province-level incidence percent change to describe temporal and spatial trends, including pre/post COVID-19 comparisons. Measles case counts showed seasonal peaks of varying intensity throughout most of 2014-2023. Meningitis cases declined in the past four years. Yellow fever cases significantly increased in the past three years. Three provinces had incidence increases of over 150% between the two periods: Mandoul (2·92 to 17·67 per 100 000; 505·5%), Mayo-Kebbi Ouest (4·28 to 11·31 per 100 000; 164·4%), and Tandjilé (3·43 to 13·08 per 100 000; 281·7%), all of which are in the southern half of the country. Neonatal tetanus experienced a slight upward trend throughout the ten-year period, with a similar distribution across nearly all provinces between the two periods under examination. Malaria steadily increased throughout the ten-year period, with most provinces reporting higher incidences during the 2020-2023 period (ranging from 2·6 to 68·1 percent increases). Acute flaccid paralysis experienced increases in the past four years, with the largest incidence increases in the south, in Mandoul (3·57 to 10·20 per 100 000; 185·6%) and Tandjilé (3·31 to 9·56 per 100 000; 188·5%). Trends in the six diseases varied throughout the time period and reflected the impact of events such as the COVID-19 pandemic, climate change, and vaccination campaigns.

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SECURING GLOBAL HEALTH: CYBERSECURITY AND HEALTH DATA PROTECTION TO IMPROVE CROSS-BORDER COLLABORATION AND EQUITY IN HEALTH DATA ACCESS IN LATIN AMERICA AND THE CARIBBEAN

Fabu Athaliah Moses Stuart

Nova Southeastern University, Fort Lauderdale, FL, United States

The acceleration of digital health in Latin America and the Caribbean (LAC) presents an opportunity for disease surveillance and pandemic preparedness; progress is endangered by systemic weaknesses in cybersecurity and data governance. There is a lack of comparative analyses identifying gaps in LAC's regulatory frameworks, how they impede cross-border health data sharing and exclude marginalized populations from digital health systems. This study addresses the need for an assessment of LAC's digital health governance landscape to inform policy. The study compared regulatory environments in 4 LAC countries -with diverse landscapes and digital health maturity levels- against the framework of the European Union's (EU) General Data Protection Regulation (GDPR) as a reference framework due to its comprehensive nature, emphasis on data protection, cross-border data flow, and established standards. Data analysis was performed using thematic coding to identify patterns and discrepancies in regulatory texts. This approach allowed for an in-depth, qualitative comparison, focusing on themes in digital health: Governance, Interoperability, Enforcement and Capacity, Equity and Trust. The analysis confirmed a disparity in digital maturity. Thus, LAC's fragmented national frameworks complicate cross-border cooperation during health emergencies. The framework offers a roadmap to enhance trust and build cyber resilience tailored to the region's challenges. A key pillar addresses fragmentation, other pillars include leveraging model laws of OAS and CARICOM, mandatory health-specific cybersecurity standards, and sustainable capacity-building funds for under-resourced environments. This framework is essential for securing global health and health equity across the region. This study's scope was limited to a comparative analysis in 4 LAC countries. While diverse, this may not represent the entire region. Future research could expand the country sample and delve into implementation challenges at the operational level. The use of EU frameworks present certain contextual differences that warrant consideration.

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ASSESSMENT OF KNOWLEDGE, ATTITUDES, AND PRACTICES REGARDING CHOLERA AMONG OUTPATIENTS AT THE K-SOLEIL CENTER IN GONAÏVES AMID THE CHOLERA REEMERGENCE IN HAÏTI, MARCH-APRIL 2024

Rose Béracaël Jarbath[1], Stéphanie Petit-Frère[1], Emmanuel Fabrice Julceus[2], Eddie Charles[2]

[1]Mount Everest University of Haiti, Port-au-Prince, Haiti, [2]Center for Integral Health and Research, Tabarre, Haiti

The reemergence of cholera cases raises fears of the disease becoming endemic in Haiti. The adoption of prevention practices depends on the level of knowledge of a disease and appropriate attitudes. Our objective was to assess the knowledge, attitudes, and practices regarding cholera among patients at general and prenatal outpatient clinics of the K-Soleil Center in Gonaïves, Haiti. This cross-sectional study included 400 consecutive patients aged 15 years and older seen at K-Soleil Center's general and prenatal outpatient clinics between March and April 2024; a sample of 363 patients was required to have 95% confidence in the proportions. Sociodemographic characteristics, knowledge, attitudes, and practices collected from a questionnaire were analyzed using counts, percentages, means, standard deviations, and the chi-square test via Epi Info 7. The mean age of the participants was 34.6 ÷ 13.7 years, 67.8% were women, 94.9% lived in urban areas, 82.0% had completed secondary education or higher, and 25.0% were professionals. The respondents' main source of information was the media (47.8%). Overall, 59.8% of respondents had a fair level of knowledge, 64.5% had appropriate attitudes, and 62.8% had appropriate practices. Respondents from urban areas, those with a secondary education or higher, and professionals were more likely to have a good/fair level of knowledge, appropriate attitudes, and correct/appropriate practices compared to their counterparts (all $p < 0.05$). A good/fair level of knowledge was associated with appropriate attitudes (69.1% vs. 55.0%, $p = 0.005$) and correct/appropriate practices (73.2% vs. 44.3%, $p = 0.00001$), compared to an insufficient/poor level of knowledge. In conclusion, more than a third of participants had inadequate knowledge, attitudes, and practices regarding cholera; geographic location, education level, and occupation were associated with these factors. Continued strengthening of health education and interventions related to water, sanitation, and hygiene, tailored to the highest-risk groups, will be essential to improving the impact of cholera control efforts.

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BEYOND THRESHOLDS: INVESTIGATING POSITIVE BLACKFLY PCR RESULTS AMIDST ZERO OV16 SEROPREVALENCE IN FCT ABUJA'S ONCHOCERCIASIS PROGRAMME

Juliana Ajuma Amanyi-Enegela[1], Joseph Kumbur[2], Rinpan Ishaya[3], Faizah Okunade[2], Enan William Adamani[2], Moses Aderogba[4], Louise Makau-Barasa[5], Chukwuemeka Cletus Makata[6], Girija Sankar[7]

[1]CBM, Cambridge, United Kingdom, [2]CBM, Abuja, Nigeria, [3]HANDS, Jos, Nigeria, [4]The END Fund, Abuja, Nigeria, [5]The END Fund, New York, NY, United States, [6]Federal Ministry Of Health, Nigeria, Abuja, FC, Nigeria, [7]CBM, Peachtree Corners, GA, United States

In onchocerciasis elimination programmes, stopping mass drug administration (MDA) requires concordant epidemiological and entomological evidence. WHO thresholds include Ov16 IgG4 seroprevalence in children under 10 (upper 95% CI ≤0·1%) and infective blackfly prevalence by O-150 PCR (upper 95% CI <0·05%). After 15+ ivermectin MDA rounds, FCT Abuja recorded an Ov16 seroprevalence of 0·0% in children aged 5-9, meeting the epidemiological threshold. However, only 136 Simulium damnosum s.l. flies were caught (2·3% of the WHO minimum of 6,000), and infective larval prevalence exceeded 0·05% in Abaji and Kwali, constituting a failed entomological assessment. This discordance prompted a formal investigation. A mixed-methods study included physicochemical characterisation of 14 river sites across six area

councils; review of entomological field practices against WHO checklists; expert consultations; a cross-sectional survey of 127 key informants; and interviews with migrants in endemic communities. Water quality was broadly compatible with S. damnosum larval development. Seasonal flow variations, microhabitat selection, and urbanisation-driven habitat degradation better explained low catch yields. Episodic marginal conditions in Abaji and Kwali included flow velocities below 0·3 m/s and dissolved oxygen below 5 mg/L. With only 136 flies caught, a single infected specimen formally surpasses the WHO threshold. Expert consultation identified long-distance vector immigration from bordering states as the main biological confounder. Many migrants in hotspot areas had never received ivermectin, creating pockets of microfilaraemic hosts near breeding sites. The discordance does not indicate programme failure. A 0·0% seroprevalence in over 3,000 sentinel children confirms minimal transmission. Twice-yearly MDA in Abaji and Kwali, alongside cross-border entomological surveillance, has been recommended prior to reassessment.

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CHILD DEATH CLUSTERING IN PAKISTAN MULTI-LEVEL RISK FACTORS IDENTIFIED THROUGH CHILD HEALTH AND MORTALITY SURVEILLANCE

Mahder Tracy[1], Zoyia Hassan[2], Raheel Allana[2], Saad B. Omer[1], Abdul Momin Kazi[2], Fauzia A. Malik[1]

[1]Peter O'Donnell Jr. School of Public Health, UT Southwestern Medical Center, Dallas, TX, United States, [2]Aga Khan University, Karachi, Pakistan

Intra-household child death clustering, defined as multiple child deaths within the same household, reveals concentrated vulnerability that goes unnoticed when deaths are analyzed separately. The Child Health and Mortality Prevention Surveillance (CHAMPS) program aims to determine the causes of death among children under five, with minimally invasive tissue sampling (MITS) serving as a central component of its surveillance approach. In Pakistan, a CHAMPS site, we aimed to characterize the extent of clustering and identify the multi-level factors driving repeated child deaths within families. We conducted a mixed-methods analysis using data from the vital registry system, verbal autopsy (VA) reports, counseling summary narratives, and follow-up interviews, across three peri-urban slums of Karachi. Quantitative surveillance data were combined with qualitative narratives to identify and triangulate themes across individual, household, and health system levels. Among 195 MITS child death cases, 49 (25%) occurred in households with multiple child deaths. Mixed-methods analysis identified consistent drivers of child death clustering across multiple levels. Individual-level factors, including fatalistic beliefs, mistrust in medical institutions and interventions, and lack of transportation to health facilities constrained timely access to care, were identified. Health system barriers included inadequate quality of care, lack of critical resources (e.g., ventilators), misdiagnosis, and breakdowns in referral pathways. At the population level, infectious disease exposures (e.g., measles, dengue) and reliance on traditional birth attendants contributed to risk. Child death clustering reflects interacting determinants across individual, household, and health system levels that can be identified through integrated surveillance. These findings offer actionable insights for identifying high-risk households and implementing targeted, equity-focused child survival strategies in policy and practice.

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PERFORMANCE AND GAPS IN NIGERIA'S ANIMAL RABIES SURVEILLANCE SYSTEM: A NATIONAL EVALUATION, 2021-2025

Dupe Arinola Hambolu[1], Taiwo I. Olasoju[2], Aishat B. Usman[3], Okoro A. Nwenyi[4], Chinyere I. Akujobi[2], Kaze J. Abok[5], Sunday E. Hambolu[5], Samuel Anzaku[2], Ogunbode O. Olanrewaju[1], Muhammad S. Balogun[6]

[1]Nigerian Field Epidemiology and Laboratory Training Program, Abuja, Nigeria, Ebitu Ukiwe Street, Nigeria, [2]Federal Ministry of Livestock Development, Nigeria, Abuja, Nigeria, [3]Ecowas Regional Center for Surveillance and Disease Control West African Health Organization, Abuja, Nigeria, [4]Africa Centres for Disease Control and Prevention (Africa CDC), Abuja, Nigeria, [5]National Veterinary Research Institute, Vom, Jos, Nigeria, Ebitu Ukiwe Street, Nigeria, [6]African Field Epidemiology Network, Abuja, Nigeria

Rabies remains endemic in Nigeria and continues to pose a significant public health burden despite the availability of effective prevention strategies. Effective surveillance is critical for monitoring disease trends and guiding control efforts. This study evaluated the performance of the national animal rabies surveillance system within the National Animal Disease Information System (NADIS) using the Centers for Disease Control and Prevention surveillance evaluation framework. A mixed-methods approach was employed, combining analysis of surveillance data from 2021–2025 with a structured survey of 224 stakeholders and key informant interviews. A total of 2,889 records were analyzed, including 735 suspected and 720 laboratory-confirmed rabies cases. The system demonstrated broad geographic coverage, with cases reported from 34 states. Flexibility was high (85.3%), and usefulness was moderate to high, with 79.5% of respondents reporting detection of new cases and a composite usefulness score of 0.692 (SD = 0.282). However, representativeness was limited at the local government level, with only 24% of LGAs reporting cases. Data quality issues included missing temporal variables and duplicate records, indicating gaps in routine data validation and use. Timeliness was variable, with delays in data collation and specimen transport. Stability was low, with 97.8% of respondents rating the system as unstable due to funding, logistical, and workforce constraints. Qualitative findings highlighted challenges in specimen transport and inconsistent feedback mechanisms, although the system supports outbreak response and One Health collaboration. These findings highlight critical gaps in surveillance system performance that may undermine progress toward national and global rabies elimination targets. Overall, the surveillance system is functional and useful but requires strengthening of data systems, laboratory access, and operational capacity to support rabies elimination efforts.

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ASSOCIATION BETWEEN TICKS AND WILDLIFE SPECIES FROM DIVERSE GEOGRAPHIC REGIONS OF COSTA RICA AND PRELIMINARY DETECTION OF *ORTHOFLAVIVIRUS* AND JINGMEN TICK VIRUS (JMTV)

Joban Quesada[1], Luis Enrique Chaves-González[1], Tatiana Murillo Corrales[1], Diana Rojas-Araya[1], Santiago García-Dobles[2], Isabel Hagnauer-Barrantes[3], Andrés Sáenz-Bräutigam[2], Adriana Troyo[1]

[1]Centro de Investigación en Enfermedades Tropicales, Universidad de Costa Rica, San José, Costa Rica, [2]Toucan Rescue Ranch, Heredia, Costa Rica, [3]Rescate Wildlife Rescue Center, Alajuela, Costa Rica

Ticks are arthropods of major public health importance due to their role as vectors of a wide range of pathogens. Costa Rica harbors high vertebrate diversity and at least 45 reported tick species, representing a valuable setting for the study of tick-borne pathogens. This study evaluated tick distribution, host associations, and viral detection in ticks collected from wildlife rescue centers across the country. Live ticks were morphologically identified using taxonomic keys and pooled according to species and host. Molecular confirmation was performed on a subset of representative specimens through partial amplification of the mitochondrial *16S rRNA* gene. *Orthoflavivirus* screening was done by RT-PCR targeting the *NS5* gene in 50 pooled samples, and metagenomic amplicon sequencing was performed for positive samples using CZID pipeline for sequence processing and taxonomical assignation. In addition, a Jingmen tick virus (JMTV)-specific end-point PCR targeting the RNA-dependent RNA polymerase gene was optimized. In total, 414 ticks (17 species) were obtained from 97 hosts (26 species), of which 216 were selected for viral screening and pooled. *Amblyomma* was the predominant tick genus (371/414, 80% of tick pools), followed by *Dermacentor* (23/414, 6%), *Ixodes* (12/414, 10%), and *Rhipicephalus* (8/414, 4%). Host-wise, *Choloepus hoffmanni* and *Iguana iguana* rendered the highest tick numbers, with 66/414 ticks (8 pools) and 60/414 ticks (3 pools), respectively. Host-associated species count was greatest in *Leopardus pardalis* (6 species) and *C. hoffmanni* and *Tapirus bairdii* (both with 3 species). Positivity for

Orthoflavivirus was 10% (5/50), and sequences yielding low coverage but high identity to multiple flaviviruses were obtained for two samples. NGS revealed amplicon similarity to Guapiaçu virus in one pool. No JMTV positive pools were detected. These findings highlight the importance of further understanding tick-host interactions and support the application of molecular tools for the study of tick-associated viruses in wildlife and human-associated environments, particularly in understudied regions such as Central America.

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DIVERSITY OF TICK SPECIES AND DETECTION OF TICK-BORNE BACTERIAL PATHOGENS IN TICKS COLLECTED FROM DIVERSE ECOLOGIES IN KENYA

Janet Ambale[1], JACOB Ngugi[1], Jane Wambui Thiiru[2], Gladys C. Kerich[3], Santos J. Yalwala[4], David Ochieng Oullo[5], Nicholas Okoth Odemba[6], Lucy Isabella[1], Cynthia Ojino[1], Paul Kyalo[1], Irene Wanjiru Miringu[2], Elizabeth Kioko[7], Joel Lutomiah[8], John Eads[9]

[1]USAMRDA-K, Kisumu, Kenya, [2]Kisumu, Kenya, [3]kemri, Kismu, Kenya, [4]US Army Med Res Unit-Kenya, Kisumu, Kenya, [5]HJFMRI, Kisumu, Kenya, [6]USAMRDA - K, Kisumu, Kenya, [7]USAMRU-K (WALTER REED PROJECT), Kisumu, Kenya, [8]Kenya Medical Research Institute (KEMRI), Nairobi, Kenya, [9]DPO, AE, United States

Ticks are vectors for bacterial pathogens of great public health significance, including members of the genera *Borrelia, Rickettsia, Anaplasma* and *Ehrlichia*; responsible for Lyme disease and relapsing fever, rickettsialpox, anaplasmosis and ehrlichiosis respectively. We conducted entomological surveillance to identify tick species diversity and associated tick-borne bacterial pathogens circulating in diverse ecologies in Kenya. Ticks were sampled from livestock and vegetation from 24.02-8.03.2026 by animal grooming and dragging respectively; morphologically identified to species, pooled, processed for DNA extraction, PCR of the 16S rRNA V3-V4 regions and sequencing using MinION MK1B. Resultant reads were analyzed using CLI EPI2ME. N=156 ticks were collected; Nairobi (n=98) and Laikipia (n=58) and identified to *Rhipicephalus evertsi evertsi* (n=61), *Rh. appendiculatus* (n=3) and *Amblyomma variegatum* (n=2*)* (Nairobi) and *Haemaphysalis leachi* (n=8) from Laikipia*. Rh. microplus* was collected in Nairobi (n=32) and Laikipia (n=50). Overall, most ticks were collected from cattle (n=74), sheep (n=72) compared to goats (n=2), and dragging n=8 ticks. The 26 pools tested (Nairobi n=18; Laikipia n=8) yielded zoonotic bacterial pathogens including *Anaplasma phagocytophilum* in a pool of *Rh. e. evertsi* from sheep in Nairobi and *Rh. microplus* from cattle in Laikipia, *Borrelia puertoricensis and B. hermsii,* in two pools of *Rh. microplus* from Laikipia, *E. chaffeensis* and *E. minasensis* from two pools of *Rh. microplus* from Laikipia and Nairobi respectively*,* and *E. ewingii* in *H. leachi* from Laikipia on vegetation. *A. phagocytophilum* causes anaplasmosis, the 2 *Borrelia* species (relapsing fever) and the 3 *Ehrlichia* species (ehrlichiosis).These findings highlight significant contribution of different ecologies to tick species diversity and associated bacterial pathogen distribution, underscoring the significance of One-Health approach to surveillance, control and management of bacterial zoonoses, especially in densely populated areas like Nairobi city where human-livestock-wildlife interaction occurs frequently.

7043

HABITAT SUITABILITY OF THREE TICK SPECIES FROM MONGOLIA FROM 2019-2024: *DERMACENTOR NUTTALLI, HYALOMMA ASIATICUM,* AND *IXODES PERSULCATUS*

Nora Cleary[1], Bazartseren Boldbaatar[2], Jodi Fiorenzano[3], Ian Alexander Pshea-Smith[4], Altantogtokh Doniddemberel[5], Erica Lindroth[6], Silas Davidson[7], B. Katherine Poole-Smith[8], Jeffrey Hertz[9], Bandikhuu Amgalanbayar[10], Irina Etobayeva[9], Adam R. Salyer[11], Andrew G. Letizia[12], Sadie Jane Ryan[1], Michael Emery von Fricken[13]

[1]University of Florida, Gainesville, FL, United States, [2]Mongolian University of Life Sciences, Ulaanbaatar, 47, Mongolia, [3]Navy Medical Research Unit TWO, FPO, Singapore, [4]University of Michigan, Burlington, MI, United States, [5]Ulaanbaatar, Mongolia, [6]AFPMB, Silver Spring, MD, United States, [7]APO, AP, United States, [8]Defense Health Agency, Fairfax, VA, United States, [9]Naval Medical Research Unit Indo Pacific, Singapore, Singapore, [10]National Center for Zoonotic Diseases, Ulaanbaatar, Mongolia, [11]Naval Medical Research Unit Indo Pacific, Sembawang, Singapore, [12]FPO, AP, United States, [13]University of Florida, Dept. of Environmental & Global Health, Gainesville, FL, United States

Identification of vector distribution in association with pathogen presence is critical to generating predictive medical intelligence and preserving readiness of military forces operating or training in Mongolia. Three key vectors of tick-borne pathogens (TBP) in Mongolia are *Dermacentor nuttalli, Ixodes persulcatus,* and *Hyalomma asiaticum* which harbor and transmit a variety of pathogens including *Anaplasma spp, Borrelia spp*, *Coxiella burnetii, Ehrlichia muris, Francisella persica, Rickettsia spp,* Crimean-Congo hemorrhagic fever virus, and Tick-borne encephalitis virus. A total of 25,400 ticks belonging to these three species were collected from 2019 to 2024 via flagging and dragging in 21 aimags in Mongolia. Habitat suitability (scale 0-1) was assessed using Boosted Regression Tree modeling of the Mongolian landscape as a function of environmental drivers, including temperature, precipitation, humidity, land cover, and soil composition which are known to influence the ecologic niches for each of the three tick species. Annual mean temperature was identified as the driver with the highest relative importance for *D. nuttalli* and *H. asiaticum*, and soil organic carbon for *I. persulcatus*. Using a suitability cutoff of >0.5, the predicted suitable area for *D. nuttalli* spans 33.2% of the country, with higher suitability in northern latitudes, especially in the northeast. The model revealed 19.2% and 4.9% suitability for *H. asiaticum* and *I. persulcatus* respectively. Understanding the ecological niche of these species as a function of environmental suitability can identify potential future risk areas for TBPs and inform targeted surveillance. This predictive forecasting tool enables military medical planners to strategically allocate resources and provide optimized force health protection guidance in pre-deployment training.

7044

DENGUE VECTOR SURVEILLANCE IN THE COASTAL REGION OF KENYA (2016-2025): SPATIO-TEMPORAL DISTRIBUTION AND RELATIVE ABUNDANCE OF *AEDES AEGYPTI*

David Oullo[1], Yalwala Sanctos[1], Nicholas Odemba[1], Gladys Kerich[1], Janet Ambale[1], Lucy Isabella[1], Jane Wambui Thiiru[1], Francis Ngere[1], Irene Miringu[1], Elizabeth Kioko[1], Joel Lutomiah[2], John Eads[1]

[1]Walter Reed Army Institute of Research- Africa, Kisumu, Kenya, [2]Kenya Medical Research Institute (KEMRI), Nairobi, Kenya

Kenyan coastal counties that form the coastal strip: Mombasa, Lamu, Kilifi, Tana River and Kwale, are prone to dengue and chikungunya transmission. The first dengue outbreak was reported in Kilifi (1982) and later in Mombasa (2013-2014). Currently, Mombasa and Lamu experience frequent dengue and chikungunya outbreaks which occur alternately. Knowledge of Aedes aegypti vector distribution and densities is crucial for assessing disease risk. From 2016-2025, we conducted entomological surveillance in Lamu, Kwale, Mombasa and Kilifi during long rainy (May-June) and dry (February) seasons to establish spatio-temporal vector distribution and relative abundance, a critical dataset for decision-making on disease monitoring for early warning, preparedness and targeted preventive measures. Mosquitoes were sampled using standard methods: CDC Light Trap, BG sentinel traps and larval collection which were reared to adults and morphologically identified to species. The same number of traps and trapping nights were applied across sites except Mombasa which had shorter collection period (2022-2025). Over 18,500 Aedes aegypti were collected across the four counties [during wet season (n=14,539) and dry season (n=4,236)]; with Lamu recording the highest collection (n=9,733 [n=6,976, (n=2,157]), Kilifi (n=3,247 [n=2,974, n=273]), Kwale (n=3,129 [n=2,427, n=702]) while Mombasa recorded the least (n=2,666 [n=2,162, n=504] during wet and dry seasons respectively. Larval survey collected the highest No. of mosquitoes (n=9,799) compared to CDC LT (n=7,096) and BG sentinel collected (n=1,880). Ae. aegypti is widely distributed in coastal region with varying levels of abundance. Seasonality is a critical determinant

of vector abundance, which may be associated with disease risk. The low numbers recorded in Mombasa reflect fewer sampling years compared to Lamu. Dengue and chikungunya outbreaks in Mombasa and Lamu are probably due to high vector densities but most importantly these mosquito populations are more competent vectors and prone to feed on humans thus increasing vectorial capacity and disease risk.

7045

SURVEILLANCE OF *HAEMAPHYSALIS LONGICORNIS* IN THE BALTIMORE REGION

Margaret V. Becker, Reneé LMN Ali, Shelby M. Cagle, Douglas E. Norris

Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

Haemaphysalis longicornis Neumann (Ixodida: Ixodidae), known commonly as the Asian longhorned tick, is an invasive tick species originally native to east and central Asia. The presence of *H. longicornis* in the United States was first reported in New Jersey in 2017, however, testing of archival samples indicated that this tick species was introduced as early as 2010. Currently, it has been documented in over 20 states with significant infestation throughout most of the eastern US. In its native territory, *H. longicornis* is a vector of several human and animal pathogens including groups of microorganisms that are known to circulate in the United States such as *Theileria orientalis* and spotted fever group *Rickettsia* species. Reports of *H. longicornis* infestations near cattle with positive cases of Theileriosis indicate the threat this tick species poses to the livestock and dairy industry in the US. While to date pathogen transmission to humans has not been documented in the US, *H. longicornis* distribution needs to be closely monitored given its potential to transmit zoonotic pathogens, which may place human populations at risk. An infestation of this tick was reported at Cylburn Arboretum, a public park in Baltimore City, in 2025. To assess the extent of the infestation, we collected ticks over two months in the fall along three transects on the property. Most of the ticks collected on both trips were *H. longicornis* larvae with counts increasing the further south we collected in the park. We sequenced the mitogenomes of an adult tick from this site and an adult collected from nearby Howard County, MD for phylogenetic analysis. This analysis demonstrated that these two *H. longicornis* specimens clustered with other ticks from eastern US suggesting that they are from the same invasive lineage. This is supported by 99.84% sequence identity with other *H. longicornis* from the US as well as this tick species' ability to reproduce parthenogenically. Increased surveillance and monitoring of population genetics for invasive *H. longicornis* in Baltimore and the surrounding region will inform the public and the One Health risk that this vector poses to the community.

7046

BITING MIDGE SURVEILLANCE IN OROPOUCHE FEVER TRANSMISSION AREAS IN IQUITOS, PERU

Astete Helvio[1], Luz Romero[2], Gonzalo Vazquez-Prokopec[3], Amy C. Morrison[4], Ryan Moore[1], **Gissella M. Vasquez**[1]

[1]U.S. Naval Medical Research Unit SOUTH, Bellavista, Peru, [2]Culmen International, Alexandria, VA, United States, [3]Emory University, Atlanta, GA, United States, [4]University of California Davis, Davis, CA, United States

Culicoides biting midges belonging to the family Ceratopogonidae, are small (1- 5 mm), blood-sucking dipterans of medical and veterinary importance. In the Americas, *Culicoides paraensis* is recognized as the main vector of Oropouche fever, which has been circulating since the 1990s and represents a public health problem in the Peruvian Amazon and other tropical regions. Our study aimed to evaluate the diversity, abundance, and species composition of biting midges in Oropouche fever transmission areas near Iquitos, a city located in the northeastern Peruvian Amazon. Specimens were collected using Prokopack aspirators and CDC light traps in 59 sites located in peri-urban and rural areas in or near Iquitos from October 2024 to October 2025. Specimens were identified to species using taxonomic keys. Diversity indices and spatial distribution patterns were described. Of the 17,670 biting midges collected, 99% were sampled with prokopack aspirators. Eight species were identified: *Culicoides paraensis*, *C. insinuatus*, *C. insignis*, *C. guttatus*, *C. foxi*, *C. pusillus, C. leopoldoi*, and *C. daviesi*. Biting midge relative abundance was the highest in sites south of Iquitos (64.56%) and the lowest in the northern part of the city (0.98%). The most abundant species were *C. insinuatus* (59.76%) and the Oropouche fever vector *C. paraensis* (36.47%); both were found in all sampled sites. Species diversity was high in sites to the west and east of Iquitos, and low in northern Iquitos where the Oropouche fever vector was dominant. Overall, biting midge populations were not homogeneous across Iquitos, showing a marked species spatial heterogeneity. Our results highlight spatially explicit epidemiological risk for Oropouche virus transmission, and potential alternative vectors to *C. paraensis* in the Peruvian Amazon region. Additionally, we provide evidence that biting midges can be effectively sampled with prokopack aspirators for the first time.

7047

DETECTION AND CHARACTERIZATION OF *LEISHMANIA* PARASITES IN SANDFLY VECTORS IN ISIOLO, KENYA, 2026

Irene W. Miringu[1], Jane Wambui Thiiru[2], Gladys C. Kerich[3], Janet Ambale[4], Lucy Isabella[5], Jacob Ngugi[5], Paul Kyalo[5], Cynthia Ojino[5], Santos J. Yalwala[6], David Ochieng Oullo[7], Nicholas Okoth Odemba[8], Elizabeth Kioko[9], Simon Muriu[1], Luna Kamau[10], Joel Lutomiah[11], John Eads[12], Fredrick L. Eyase[13]

[1]Pwani University, Kilifi, Kenya, [2]Kisumu, Kenya, [3]kemri, Kismu, Kenya, [4]USAMRDA-K, Kisumu, Kenya, [5]Walter Reed Army Institute of Research, Kisumu, Kenya, [6]US Army Med Res Unit-Kenya, Kisumu, Kenya, [7]HJFMRI, Kisumu, Kenya, [8]USAMRDA - K, Kisumu, Kenya, [9]USAMRU-K (WALTER REED PROJECT), Kisumu, Kenya, [10]Kenya Medical Research Institute, Nairobi, Kenya, [11]Kenya Medical Research Institute (KEMRI), Nairobi, Kenya, [12]DPO, AE, United States, [13]USAMRD-K, Nairobi, Kenya

Leishmaniasis is a significant public health concern in Kenya. It is caused by protozoan parasites of the *Leishmania* genus and transmitted by female phlebotomine sandflies. The disease presents in form of cutaneous (CL), mucocutaneous (MCL), and visceral (VL or kala-azar), with VL and CL being the most predominant in Kenya. While *Leishmania* particularly impacts arid and semi arid regions through outbreaks, there are emerging cases in new areas, probably due to climate change. This underscores the need to investigate the biodiversity and distribution of the parasites and their vectors to understand disease epidemiology and inform targeted interventions. From February 24 to March 08, 2026, sandflies were sampled in and Nairobi, Isiolo and Samburu Counties using CO2-baited CDC light traps, morphologically identified to genus level using standard taxonomic keys, pooled based on the genus and transported in dry ice to the KEMRI/WRAIR-Africa laboratory at Kisian campus, Kisumu, Kenya, for analysis. DNA was extracted from the pools using the QIAamp DNA mini kit and screened for Leishmania parasites by qPCR using Heat Shock Protein 70 (HSP70) primers and probes. Overall, n=273 sandflies were collected from Nairobi (n=15), Isiolo (n=225), and Samburu (n=33) and identified to *Phlebotomus* and *Sergentomyia* genera. *Leishmania* DNA was detected in a pool of female *Sergentomyia* sandflies collected from Isiolo County. Further molecular characterization of both the detected *Leishmania* parasite and associated sandfly vector is currently ongoing. Isiolo County is endemic for both cutaneous and visceral leishmaniasis. Although members of the *Sergentomyia spp*. have not been conclusively incriminated as vectors of human leishmaniasis, characterization of the parasite will provide insights into the *Leishmania* species circulating in the area, the potential role of *Sergentomyia spp*. in the transmission and the risk of human exposure. These findings will also inform targeted surveillance and appropriate control strategies.

7048

INVESTIGATING SMALL MOLECULE INHIBITORS TARGETING REPRODUCTIVE PROTEINS IN *ANOPHELES GAMBIAE* S.L. AS AN INNOVATIVE PATHWAY TO REDUCE MALARIA TRANSMISSION

Fatoumata Seck[1], Karim Mané[2], Moussa Diallo[2], Mouhamadou Fadel Diop[2], Ebrima SM Kolley[2], Babacar Diouf[3], Marieme Gueye[3], El Hadji Malick Ngom[3], Ibrahima Dia[3], Benoit Sessinou Assogba[2]

[1]MRCG@LSHTM, IPD, African Centre of Excellence in Biotechnological Innovations for Vector-borne Diseases Elimination (CEA ITECH-MTV), Université Nazi Boni, Burkina Faso, [2]Medical Research Council Unit, The Gambia at London School of Hygiene and Tropical Medicine (MRCG@ LSHTM), Banjul, Gambia, [3]Institut Pasteur of Dakar (IPD), Dakar, Senegal

Targeting key genes involved in mosquito fertility and susceptibility to *Plasmodium* using small molecule inhibitors represents a promising alternative approach to reduce malaria transmission. In this study, we investigated four candidate genes (*MISO*, *HPX15*, *VG* and *LP*) associated with *Anopheles* reproductive success and vector competence in *Anopheles gambiae* s.l. Using whole-genome SNP data from the Ag1000G project, we analyzed these genes in 2,784 wild-caught *Anopheles gambiae* s.l. collected across 19 sub-Saharan African countries. Population genetic analyses were conducted to assess structure, genetic diversity and differentiation, alongside neutrality tests and evaluation of nonsynonymous SNP (nsSNP) linkage. Our results revealed a high level of conservation across all four genes in natural populations, suggesting their essential biological roles and potential as robust molecular targets. These findings provide integrated insights into key reproductive and transmission-related traits of major malaria vectors within their natural genetic background, supporting the development of innovative and sustainable vector control strategies. To further explore functional implications, *HPX15* was selected for experimental validation. Four candidate small molecules were tested for their impact on *Anopheles coluzzii* life-history traits. While no significant effects were observed on mating or insemination, inhibition of *HPX15* disrupted critical physiological processes, including reduced fecundity, decreased embryonic viability and shortened adult lifespan. The combined impact on reproductive success and survival highlights the strong epidemiological relevance of this approach. Importantly, targeting highly conserved biological functions may reduce the likelihood of rapid resistance emergence compared to conventional insecticide-based strategies that act on more adaptable traits.

7049

COMPUTER VISION BASED DIFFERENTIATION OF CRYPTIC MALARIA VECTORS ANOPHELES ARABIENSIS AND ANOPHELES COLUZZII

Samia Kazemi[1], Roy Faiman[1], Zana Lamissa Sanogo[2], Mamou Sanogo[2], Alassane Dit Assitoun[3], Moussa M. Diallo[4], Hannah L. Markle[5], Khushi Anil Gupta[1], Autumn Goodwin[6], Vasiliki Ikonomidou[1], Thomas Jenkins[1]

[1]VecTech, Baltimore, MD, United States, [2]ICER Mali, Bamako, Mali, [3]Bamako, Mali, [4]ICERMALI, Bamako, Mali, [5]Vectech, Baltimore, MD, United States, [6]Baltimore, MD, United States

Accurate species-level identification within the *Anopheles gambiae* complex is critical for malaria vector control, as member species differ substantially in ecology, host preference, and vectorial capacity despite near-identical morphology. Current field identification typically groups these species, and molecular differentiation is the gold standard, limiting the precision of surveillance and intervention strategies. This study evaluated the potential for a computer vision based system (IDX, Vectech, Inc.) to differentiate *An. arabiensis* from *An. coluzzii* morphologically. Field-collected male and female specimens of both species (175 *An. arabiensis* and 396 *An. coluzzii*) were obtained from two sites in Mali — Bamako and Sikasso — and molecularly confirmed via ITS2 at Mali's ICER program, with species identity blinded at time of imaging to reduce label bias. Two experimental phases were conducted using the IDX v6.0 algorithm which implements a classifier based on the Xception architecture. Phase 1 of our experiments involves a two-class fine-tuning of a model pretrained on the larger IDX v6.0 dataset with 77 mosquito species, using only the PCR-labeled specimens from the two target species. Phase 2 consists of an 18-class multi-species training phase incorporating additional Anopheles data with specimen masking applied. Phase 1, averaged recall remained above chance for both species (*An. arabiensis*: 68 ± 4%; *An. coluzzii*: 76 ± 11%), with consistent error distributions across collection sites indicating the model was learning morphological rather than background signals. In phase 2, the best-performing model achieved recall of 77.8% (95% CI: 59.2-89.4%) for *An. arabiensis* and 58.5% (95% CI: 45.1-70.7%) for *An. coluzzii*, with grad-CAM attention maps showing improved focus on mosquito body features when masking was applied. These preliminary results suggest that CV-based sub-complex species separation of cryptic malaria vectors may be feasible and warrants further investment in larger, geographically diverse, PCR-labeled training datasets and advanced feature extraction approaches to achieve field-deployable accuracy.

7050

LARGE-SCALE CAPACITY BUILDING FOR LARVAL SOURCE MANAGEMENT IN TANZANIA: A COST-EFFICIENT NATIONWIDE TRAINING OF 740 IMPLEMENTERS

Jubilate Bernard Minja[1], Charles Dismas Mwalimu[2], Edward Sambu[3], Bernard Malongo[3], Rosemary Nshama[4], Denis Kailembo[5], Tegemeo Gavana[6], Stella Kajange[7], Best Yoram[7], Noela Kisoka[8], Johnson Matowo[9], Yahya Athumani Derua[10]

[1]KCMC University, MOSHI, United Republic of Tanzania, [2]national malaria control progamme, Dar Es Salaam, United Republic of Tanzania, [3]National Institute for Medical Research- Amani, Tanga, United Republic of Tanzania, [4]National Malaria Control Programme, Dodoma, United Republic of Tanzania, [5]Dar Es Salaam, United Republic of Tanzania, [6]SwissTPH / University of Basel, Basel, Switzerland, [7]Prime Minister's Office- Regional Administration and Local Government, Dodoma, United Republic of Tanzania, [8]Swiss TPH- TEMT, Dar es Salaam, United Republic of Tanzania, [9]Kilimanjaro Christian Med Univ College, Moshi, United Republic of Tanzania, [10]Moshi, United Republic of Tanzania

Malaria has remained high in some regions of Tanzania despite existing control interventions. The TDHS of 2022, reported an average prevalence of 8.1%, with wide geographical variation. Persistent transmission is linked to insecticide resistance, change in mosquito behaviour, drug resistance, and community-level factors. To address this challenge, larval source management was prioritized in the National Strategic Plan for Malaria elimination by 2030. However, implementation of LSM is limited by inadequate skills of implementers, prompting NMCP and partners to organized a training to build LSM implementation capacity at district and regional levels. The main objective of the training was to develop a core group of competent regional and council-level trainers to effectively plan, train sub-council teams, coordinate implementation, and supervise scale-up of the LSM intervention according to national guidelines. A total of 740 participants from 179 councils and 25 regions attended the training by experienced facilitators, in eight centres distributed countrywide. The structure of the training combined two days of virtual theory and two days of field practicals. Moreover, knowledge acquisition and the general conduct of the training were evaluated by using the end-of-training examination and an evaluation questionnaire. This interactive programme covered skills in planning, larval surveillance, source reduction through environmental management, larviciding, supervision, monitoring, evaluation, and reporting. Acquired knowledge and training quality were assessed through examination and questionnaire, respectively, showing high knowledge retention and overall participant satisfaction, with ongoing mentorship planned to support effective LSM implementation, towards malaria elimination in Tanzania by 2030.

7051

BACTERIA FROM THE *ANOPHELES DARLINGI* MIDGUT MICROBIOTA WITH POTENTIAL TO INHIBIT *PLASMODIUM VIVAX*

Estefani Piedrahita[1], Adriana Rubio[1], Santiago Pemberty[1], Najara AC. Santos[2], Jessica EA. Kassupá[2], Alessandra S. Bastos[2], Eliza M. Costa[2], Maisa S. Araujo[2], Margarita M. Correa[1]

[1]Grupo Microbiología Molecular, Escuela de Microbiología, Universidad de Antioquia, Medellín, Colombia, [2]Plataforma de Produção e Infecção de Vetores de Malária (PIVEM), Fiocruz, Rondônia, Brazil

Malaria control is threatened by increased mosquito resistance to insecticides, making it necessary to propose new vector control strategies, such as those based on microorganisms. It is known that bacteria of the *Anopheles* midgut microbiota can affect *Plasmodium* parasite development; therefore, this study aimed to evaluate the effect of *Enterobacter* sp. and *Serratia* sp., isolated from *Anopheles* spp. microbiota, on *Anopheles darlingi* vector competence. Female *An. darlingi* mosquitoes aged 3-5 days were independently treated with each bacterium and their supernatants, and subsequently infected with *Plasmodium vivax*. The bacteria impact on parasite development was evaluated by counts of the ookinete, oocyst, and sporozoite forms under optical microscopy and immunofluorescence post infection at 21 hours, 7 days, and 14 days respectively. In addition, bacterial impact on vector survival was evaluated. Both bacteria, *Enterobacter* sp. and *Serratia* sp., showed potential to inhibit parasite development in *An. darlingi* infected with *P. vivax*. Specifically, *Serratia* sp. supernatant significantly decrease *P. vivax* sporozoites. Furthermore, *Serratia* sp. treatment affected vector survival. These findings suggest that mosquito-associated bacteria could be used as a complementary approach to malaria control.

7052

OPERATIONALIZING ENVIRONMENTAL DNA (EDNA) DETECTION OF MAJOR MALARIA VECTOR SPECIES IN GHANA

Mojca Kristan[1], Yaw Asare Afrane[2], Yaw Akuamoah-Boateng[3], Abdul Rahim Mohammed Sabtiu[4], Judith D. Azumah[5], Anisa Abdulai[4], Issac K. Sraku[5], Bright C. Obeng[5], Karen Lorena Figueroa Chilito[6], Jo Lines[7], Sian E. Clarke[8], Taane Gregory Clark[7], Susana Campino[9], **Louisa Alexandra Messenger**[10]

[1]London School of Hygiene & Tropical Medicine, Coulsdon, United Kingdom, [2]University of Ghana Medical School, Accra, Ghana, [3]Accra, Ghana, [4]university Of Ghana, Accra, Ghana, [5]University of Ghana, Accra, Ghana, [6]Henderson, NV, United States, [7]London, United Kingdom, [8]London Sch of Hygiene & Tropical Medicine, London, United Kingdom, [9]London School of Hygiene and Tropical Medicine, London, United Kingdom, [10]University of Nevada, Las Vegas, Henderson, NV, United States

The global landscape of vector-borne diseases (VBD) is shifting, driven by climate change, urbanization, and widespread habitat modification, requiring novel approaches to surveillance, prevention and control. To improve our capacity to respond to real-time emergent VBD threats, this study undertook cross-sectional sampling of suspected mosquito breeding sites in Greater Accra and Western Region, Ghana, to assess the operational feasibility of environmental DNA (eDNA) detection of major malaria vector species. eDNA deposited by wild, immature *Anopheles gambiae* s.l., isolated from a 50 ml water sample, was detectable using both end-point PCR and qPCR, achieving high levels of sensitivity (76.28%) and specificity (100%). Furthermore, we recovered PCR amplicons covering eight known insecticide resistance mutations from eDNA collected in natural breeding sites. This study represents the first proof-of-concept demonstrating that eDNA can be used for the simultaneous identification of *Anopheles* vectors and circulating insecticide resistance alleles directly from aquatic habitats in the community, including the first report of *gste2*-F120L in Ghana. Our eDNA sampling methodology was designed for citizen scientists as end-point users. We recommend the use of fixed filters sampling 50 ml of water as a highly feasible, sensitive and specific, community-led mosquito vector surveillance tool, which requires minimal training or technical infrastructure.

7053

STUDY OF SPECIES COMPOSITION REVEALED AN UNEXPECTED PRESENCE OF *ANOPHELES COLUZZII* IN A SOUTHERN AREA OF MOZAMBIQUE

Sintia Layla Tamele[1], Sheila Nhate Macamo[2], Ayesha Siddikah Aswat[3], Alberto Filipe Machava[1], Dulcisária Marrenjo[4], Celso Alafo[1], Khalid Ussene Bapú[1], Luís Fortunato[1], Kiri Rundle[5], Roshanta Dhanai[6], Francois Maartens[7], Sherwin Charles[8], Nelson Jacinto Cuamba[1]

[1]Fundação Goodbye Malaria, Boane, Mozambique, [2]Johannesburg, South Africa, [3]Wits Research Institute for Malaria, Johannesburg, South Africa, [4]National Malaria Control Program, Maputo, Mozambique, [5]Goodbye Malaria, Johannesburg, South Africa, [6]Lubombo Spatial Initiative Development 2 NPC, Johannesburg, South Africa, [7]Lubombo Spatial Initiative Development 2 NPC, Durban, South Africa, [8]Goodbye Malaria, Johannesburg, GP, South Africa

Four members of the *Anopheles gambiae* complex are known to occur in Mozambique: *An. gambiae sensu stricto (s.s.)*, *An. arabiensis* and *An. merus*, which are involved to varying degrees in malaria transmission, and the non-malaria vector *An. quandriannulatus*, each exhibiting different geographical distribution and behaviour characteristics. This study aimed to study species composition and their infection rates status in Maputo province, an area target for malaria elimination. Mosquitoes were collected from 2020 to 2024 in Maputo province, in 8 sentinel sites of National Malaria Control Program (NMCP) using standard entomological collection techniques such as Human Landing Catches (HLC), CDC Light Traps (CDC LT) and Pyrethrum Spray Catches (PSC). Species were identified by Polymerase Chain Reaction (PCR) methods. *Plasmodium* infections were analyzed through PCR and an Enzyme-linked Immunosorbent Assay (ELISA). During 2021, three *Anopheles coluzzii* specimens (1.66%) from Boane, Manhiça and Matola were identified out of 181 *An. gambiae* complex specimens. This constitutes the first reporting of *An. coluzzii* in Mozambique and in the southernmost region of Africa. However, analyses conducted in collection of 2022 to 2024 did not detect this species and only confirmed that *An. merus* and *An. funestus* s.s. are the main members of *An. gambiae* complex and *An. funestus* group, respectively. Infection analyses did not detect the presence of sporozoites in *An. coluzzii*. The only species found infected were *An. arabiensis*, *An. gambiae s.s.*, *An. merus* and *An. funestus s.s.*. *An. coluzzii*, initially found in West Africa, extended its distribution beyond this region, including East Africa, but has not been reported in the most southern regions of Africa. The presence of *An. coluzzii* in the southern areas of Mozambique was an important but unexpected finding. Further work is recommended on the distribution of the members of this complex, sequencing the samples to confirm the species as well as vector incrimination in various locations in the country and region.

7054

CONTRASTING ENVIRONMENTAL DRIVERS OF *ANOPHELES FUNESTUS* AND *AN. GAMBIAE* LARVAL ABUNDANCE IN KILOMBERO VALLEY, TANZANIA

Yohana Mwalugelo[1], Dickson Samwel Msaky[1], Herieth Hezron Mahenge[2], Polius Gerazi Pinda[2], Joel Nkya[2], Mohamed Jumanne[1], Paul Calist Mrosso[3], Reuben Elias Mmweteni[4], Khamis Kifungo[2], Hamis Hussein Bwanaly[5], Letus Laurian Muyaga[2], Douglas E. Norris[6], Michael Reddy[7], Isaiah Hoyer[7], Halfan Said Ngowo[8], Issa Hassani Mshani[2], Fredros Oketch Okumu[8], Emmanuel Willson Kaindoa[9]

[1]Ifakara Health Institute, Dar Es Salaam, United Republic of Tanzania, [2]Ifakara Health Institute, Morogoro, United Republic of Tanzania, [3]Morogoro, United Republic of Tanzania, [4]Morogoro, United Republic of Tanzania, [56]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United

States, [7]Microsoft Research, Redmond, WA, United States, [8]Ifakara Health Institute, Ifakara, United Republic of Tanzania, [9]Ifakara Health Inst, Ifakara, United Republic of Tanzania

Understanding larval ecology of malaria vectors is essential for optimizing larval source management. However, species-specific environmental drivers of larval abundance remain poorly understood, particularly for *Anopheles funestus* and *Anopheles gambiae,* which occupy distinct ecological niches but are rarely compared under natural field conditions. This study quantified habitat characteristics and environmental predictors of larval abundance of both species in the Kilombero Valley, Tanzania. A longitudinal grid-based surveillance system covering 62 grids (15×15 km) was implemented, with up to 600 aquatic habitats characterized and sampled monthly from March 2024 to December 2025. Larvae were collected using standard dippers or 10L buckets and morphologically identified using standard taxonomic keys; *Anopheles funestus* sensu lato and *Anopheles gambiae* sensu lato were included in analysis. Habitat characteristics were recorded, including physicochemical variables and physical features. Associations between environmental factors and larval abundance were analyzed using negative binomial generalized linear mixed-effects models. For *An. funestus s.l.*, abundance increased with higher pH (RR=1.14, $p < 0.001$), vegetation (RR=1.60, $p < 0.001$), algae (RR=1.39, $p < 0.001$), shade (RR=1.24, $p < 0.001$), and slow-moving water (RR=1.73, $p < 0.001$), but decreased with increasing temperature (RR=0.89, $p < 0.001$) and turbidity (RR = 0.32, $p < 0.001$). In contrast, *An. gambiae s.l.* abundance increased with temperature (RR=1.16, $p < 0.001$), temporary habitats (RR=1.24, $p < 0.001$), and turbidity (RR=1.67, $p < 0.001$), but decreased in shaded, vegetated, and algae-rich habitats (RR=0.53-0.85, $p < 0.001$). These findings demonstrate clear ecological divergence between *Anopheles funestus* s.l. and *Anopheles gambiae* s.l. with distinct environmental conditions associated with larval abundance. The identified habitat characteristics highlight opportunities to refine larval source management by prioritizing habitat types that disproportionately contribute to each species without increasing operational complexity.

7055

MOSQUITO NECTAR MEAL SEQUENCING TO IMPROVE UNDERSTANDING OF VECTOR-BORNE DISEASE ECOLOGY

Mae Igwe[1], Wenqiao He[2], Jonathan Parr[1]

[1]University of North Carolina at Chapel Hill, Chapel Hill, NC, United States, [2]Chapel Hill, NC, United States

As vectors for many human pathogens, mosquitoes are a major target of infectious disease control efforts. Investigation of mosquito-plant interactions through nectar meals can provide insights into disease ecology and opportunities for targeted intervention. DNA sequencing can be used to uncover recent mosquito nectar meals, facilitated by growing databases of barcode gene sequences for the conserved chloroplast enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase's large subunit (*rbcL*). While female mosquitoes feed on blood to support egg development, both males and females depend on nectar as a core energy source. Thus, an understanding of mosquito-plant interactions may yield insights for control programs that limit mosquito breeding or blood meal frequency. However, identification to the species level has proved challenging as *rbcL* sequences often resolve only to the family or genus level. Furthermore, the trace concentration of plant DNA present in mosquito pools can be difficult to amplify sufficiently for DNA sequencing. We are optimizing methods for species-level nectar source identification using *rbcL* alongside maturase K (*matK*), another chloroplast barcode gene with greater interspecies variation, paired with Nanopore sequencing. Initial low-concentration spike-in experiments using insectary-reared mosquitoes confirm successful plant identification at the species level. In trials of pure and mixed plant mixtures, for example, we successfully identified *Heptapleurum arboricola* within a 100 ng DNA sample from the mosquito species *Culex quinquefasciatus*, in which pure *H. arboricola* constituted 0.0001% of total DNA. In a similar trial, we successfully identified both *H. arboricola* and *Fraxinus americana* from a mixed plant DNA sample constituting 0.01% of total DNA. We are currently optimizing reaction conditions and applying them to wild-caught mosquito pools from North Carolina to achieve more comprehensive nectar meal characterization. These methods will enable species-level mosquito nectar meal analysis and ultimately be used to improve our understanding of vector-borne disease ecology.

7056

ANOPHELES MOSQUITO-HOST INTERACTION AND MALARIA TRANSMISSION IN SITES WITHIN TWO ECOLOGICAL ZONES OF GHANA

Lourees Esi Awotwe

University of Ghana, Accra, Ghana

Understanding mosquito species composition, host-feeding behavior, malaria infection rates, and insecticide resistance is essential for effective vector control. This study assessed the abundance, species composition, blood meal sources, sporozoite infection, and knockdown resistance (kdr) mutations in *Anopheles gambiae* sensu lato across the Coastal Savannah and Guinea Savannah zones of Ghana. Seasonal indoor and outdoor sampling of adult mosquitoes was done using prokopack aspiration. Species identification and host blood meal origin were determined using PCR. *Plasmodium falciparum* sporozoite infection (Pf-CSP), and kdr mutations genotyping was done using PCR. A total of 20,809 mosquitoes were sampled over the sampling period: *Culex* (51.9%), *Anopheles* (44.8%), *Aedes* (2.6%) and *Mansonia* (0.7%). Species discrimination of *Anopheles gambiae* s.l. showed that, the most abundant species was *An. coluzzii* 81.2% (549/676). Blood-meal analysis of blood-fed *An. gambiae* s.l. showed that 53.6% fed on animals, 40.7% on humans and 3.4% had mixed blood meals. The overall sporozoite rate was 2.4%, with 1.9% in *An. coluzzii* and 0.3% in *An. gambiae* s.s. The kdr L1014F mutation showed a high allele frequency (0.95–1.00), while L1014S occurred at low frequency (0.00–0.17) and N1575Y was absent (0.00). These findings indicate ongoing malaria transmission, diverse host-feeding behavior, and widespread insecticide resistance, necessitating the urgent need for integrated vector control strategies in Ghana.

7057

MOSQUITO DIVERSITY, HOST-FEEDING PATTERNS, AND ARBOVIRUS SURVEILLANCE IN WESTERN VIRGINIA

Gavyn B. Sanford, Tom Stanley, Leslie Prillaman, Joanna Reinhold, Chloé Lahondère, Gillian Eastwood

Virginia Tech, Blacksburg, VA, United States

Mosquito-borne arboviruses are a growing concern in the United States, yet surveillance in rural regions remains limited. In Virginia, surveillance is targeted in urban areas of the Coastal and Piedmont regions, leaving inland counties underserved in vector monitoring and limits understanding of arboviral threats and enzootic transmission. This study addresses gaps in awareness of mosquito species diversity and arbovirus prevalence across western Virginia. Weekly mosquito collections were conducted during the summers of 2023-2025 in up to five counties along the I-81 corridor using BG-Pro, CDC light, and GAT traps deployed in urban and sylvatic habitats. Specimens were identified to species, pooled by trap type, site, and week, and screened for arboviruses using cell culture isolation methods followed by molecular identification. Blood meal analysis of engorged specimens were conducted using DNA extraction, amplification of the 16S rRNA gene, and Sanger sequencing. In 2023, 997 mosquitoes representing 18 species across 7 genera were collected, including *Aedes albopictus*, *Ae. japonicus*, and *Culex pipiens*; screened as 424 pools, West Nile virus was isolated from samples detected in Rockbridge and Rockingham counties. In 2024, 1,668 mosquitoes representing 22 species across 5 genera were collected, including several known arbovirus vectors such as *Ae. albopictus, Ae. japonicus, Ae. triseriatus, Ae. vexans, An. quadrimaculatus, An. crucians, and Ps. columbiae*. Screening the 2024 samples as 362 pools, West Nile virus and Cache Valley virus were isolated from Rockbridge County. Samples from analysis of vector community composition and arbovirus prevalence across all three years will be presented. Blood meal analysis indicates feeding on a broad range of hosts including: humans, domestic

animals, and wild animals. These findings provide the first regional catalog of mosquito diversity in Western Virginia, as well as evidence of arboviral activity in this underserved area of the state. Continued monitoring will improve understanding of vector-host-pathogen dynamics and inform local public health and vector control strategies.

7058

OUTDOOR RESTING MICROHABITATS OF *ANOPHELES* VECTORS DURING THE DRY SEASON IN URBAN-RURAL CONTINUUM OF BAMAKO, MALI

Amadou Sékou Traore[1], Mohamed Mounine Traore[1], Moussa Keita[2], Issa Traore[1], Aboubakr Sadick Kone[1], Younoussa Ziguime[1], Yaya Coulibaly[1], Gaoussou Fofana[1], Mamadou Goita[1], Bourema Cisse Bourema Cisse[1], Sidibé M'Baye Thiam[1], Cheick Oumar Tangara[1], Hamady Coulibaly[1], Fousseyni Kane[1], Mahamadou Diakite[1], Mahamoudou Toure[1], Seydou Doumbia[1], Gunter C. Muller[3], Nafomon Sogoba[2]

[1]University Clinical Research Center (UCRC) University of Sciences, Techniques and Technologies of Bamako (USTTB), Bamako, Mali, [2]Infectious Disease and Medical Entomology Research and Training Center (IDMERTC), International Center for Excellence in Research, Faculty of Medicine and Odonto-Stomatology, University of Sciences, Techniques and Technologies of Bamako (USTTB), Bamako, Mali, [3]Kuvin Center for the Study of Infectious and Tropical Diseases, Hadassah Medical School, The Hebrew University, Jerusalem, Israel

Mosquito resting sites are critical for survival, feeding, and reproduction. While the resting behavior of endophilic malaria vectors is well described, that of exophilic species is poorly understood, particularly during the dry season. This study investigated outdoor resting sites of Culicidae in urban Bamako and surrounding rural areas and assessed sugar-feeding status and *Plasmodium falciparum* infection among malaria vectors during the dry season. Potential resting habitats were sampled using Prokopack aspirators. Collected mosquitoes were morphologically identified, and members of the *Anopheles gambiae* complex were confirmed by PCR. Sugar feeding was assessed using the anthrone test, and *Plasmodium falciparum* sporozoite infection was detected using Enzyme-linked Immunosorbent Assay (ELISA). A total of 1,190 Culicidae were collected, of which 24 (2%) were *Anopheles*. Nineteen specimens belonged to the *Anopheles gambiae complex*, including four males and 15 females. Female specimens comprised four *Anopheles arabiensis* and 11 *An. coluzzii*. Sugar feeding was detected in 27% (4/15) of females, and no *Plasmodium falciparum* infections were identified. Outdoor resting microhabitats identified included tree holes, wells, rock crevices, flowers, and other natural shelters. However, only tree holes and wells harbored resting *Anopheles* mosquitoes. These findings demonstrate that Anopheles mosquitoes persist during the dry season by occupying specific outdoor microhabitats that provide favorable microclimatic refuge, and access to sugar sources. Identifying these habitats offers critical evidence for designing outdoor vector control strategies, particularly in urban and peri-urban settings where conventional indoor interventions may be less effective. These findings underscore the importance of incorporating outdoor resting behavior into malaria control programs to address residual transmission and sustain progress toward elimination.

7059

ESTIMATION OF ANOPHELES LONGEVITY IN THE FIELD WITH A MECHANISTIC MODEL OF MOSQUITO AGING

Max McClure[1], Ambrose Oruni[2], Emmanuel Arinaitwe[3], Alex K. Musiime[4], Patrick Kyagamba[3], Geoffrey Otto[3], James Adiga[3], Jackson Asiimwe Rwatooro[3], Maxwell Kilama[3], Paul Joseph Krezanoski[1], Jessica Briggs[1], Philip Rosenthal[1], Joaniter Nankabirwa[5], Moses R. Kamya[5], Grant Dorsey[1], Bryan Greenhouse[1], Isabel Rodriguez-Barraquer[1]

[1]University of California San Francisco, San Francisco, CA, United States, [2]Centre for Research in Infectious Diseases, Yaounde, Cameroon, [3]Infectious Diseases Research Collaboration, Kampala, Uganda, [4]Ministry of Health, Kampala, Uganda, [5]Makerere University, Kampala, Uganda

Assessment of *Anopheles* mosquito longevity is critical both for monitoring the effectiveness of vector control interventions and for parameterizing models of malaria transmission. A common approach to estimating mosquito survival is Davidson's parous rate method, which assumes perfect knowledge of field gonotrophic cycle length. As an alternative approach to longevity estimation that explicitly accounts for uncertainty in relevant entomological variables, we formulated a compartmental model of mosquito aging that incorporates commonly used assessments of mosquito abdominal status and parous rates. The model includes compartments for unfed, fed and gravid stages of both nulliparous and parous mosquitoes, with parous mosquitoes cycling through the three stages. Mortality rate is assumed to be constant across all compartments, and mean gonotrophic cycle length is represented by the estimated transition time between unfed compartments. To estimate parameters of interest, we are fitting this mechanistic model to previously collected entomological field data in a Bayesian framework. In these field data, *Anopheles* mosquitoes were collected from households in Busia District in Eastern Uganda using CDC light traps set overnight at two-week intervals in participating households. All captured mosquitoes were classified as unfed, fed or gravid. A random subset of mosquitoes from each capture was dissected to assess parity. We will estimate survival and gonotrophic cycle length, comparing estimates to those derived from existing methods and assessing model fit and convergence using standard MCMC diagnostics, including evaluation of Rhat, effective sample size and trace plots, posterior predictive checks, and comparison with models using alternative priors and structures using leave-one-out cross validation. This method integrates information from several entomological metrics while accounting for uncertainty in each, potentially complementing existing methods of mosquito longevity estimation without requiring changes in current entomological surveillance methods.

7060

CHARACTERIZING HOUSEHOLD MICROCLIMATE EFFECTS ON *ANOPHELES* SPECIES SPECIFIC BITING BEHAVIOR AND HUMAN BEHAVIOR ACROSS THREE DISTRICTS IN UGANDA

Marvious Kiwanuka[1], Alex Musiime[2], Daniel Basangwa[3], Perpetra Akite[1], Maxwell Kilama[4], Edward Thomsen[5], Catherine Maiteki-Sebuguzi[6], Joaniter Nankabirwa[7], Moses R. Kamya[1], Bryan Greenhouse[8], Grant Dorsey[8], Neil Lobo[9], Max McClure[10], Isabel Rodriguez-Barraquer[11], Paul Joseph Krezanoski[12]

[1]Makerere University, Kampala, Uganda, [2]National Malaria Elimination Division, Ministry of Health, Uganda, Kampala, Uganda, [3]1.Infectious Diseases Research Collaboration (IDRC), 2C Nakasero, Kampala, Uganda, [4]Kampala, Uganda, [5]5.Malaria Elimination Initiative (MEI), Institute for Global Health Sciences, University of California, San Francisco (UCSF), Mission Hall, San Francisco, CA 94143, San Francisco, CA, United States, [6]Ministry of Health, Kampala, Uganda, [7]Makerere Univ Kampala, Kampala, Uganda, [8]Univ of California San Francisco, San Francisco, CA, United States, [9]Univ of Notre Dame, South Bend, IN, United States, [10]UCSF, San Francisco,

CA, United States, [11]University of California, San Francisco, San Francisco, CA, United States, [12]University of San Francisco, California (UCSF), San Francisco, CA, United States

A growing body of evidence suggests that the microclimate in and around sleeping structures is likely to have substantial influence on how infectious mosquitoes and humans overlap in time and space. Existing studies of malaria transmission that consider these effects typically rely on remotely sensed data and fail to consider ways in which peri-domestic weather conditions may affect both mosquito and human behavior. This study aims to investigate the associations between directly measured temperature and humidity and mosquito-human overlap. As part of the ongoing Human and Vector Interaction (HAVI) entomological and human behavioral surveillance cohort, we are conducting indoor and outdoor overnight human landing catches of female *Anopheles* mosquitoes paired with observations of human behavior at 17 districts in Uganda, with six households enrolled per district. Mosquitoes are morphologically identified to the species complex level. In addition, we are deploying temperature and humidity data loggers at nine of these households across three districts in Eastern and Northern Uganda starting in April 2026, anticipating that approximately 5 months of microclimate data will available for analysis by the time of the conference. We plan to use generalized additive mixed effects models to characterize associations between microclimate variables and both insecticide-treated net use patterns and species-specific human biting rates. This pilot will provide the grounding for an expanded program of integrated environmental, entomological and human behavioral monitoring with the goal of characterizing routes of residual malaria transmission in Uganda.

7061

AEDES AEGYPTI BREEDING SITE SURVEILLANCE: TESTING THE RELIABILITY OF RATERS AND CLASSIFICATION OF DRONE-BASED TRASH MAPPING

Katherine Woo[1], Morgan Schillinger Tarpenning[2], Juliet Bramante[3], Kavita Dave Coombe[1], Andrew Chamberlain[1], Mehedy Hassan[4], Paul Mutuku[5], Donal Bisanzio[6], Giulio De Leo[7], A. Desiree LaBeaud[1], Bryson Alberto Ndenga[8], Francis Mutuku[9], Joelle I. Rosser[10]

[1]Stanford University, School of Medicine, Palo Alto, CA, United States, [2]Woodside, CA, United States, [3]University of Washington, Seattle, WA, United States, [4]Stanford University, Palo Alto, CA, United States, [5]Technical University, Mombasa, Kenya, [6]RTI International, United Kingdom, [7]Stanford University, Monterey, CA, United States, [8]Kenya Medical Research Institute, Kisumu, Kenya, [9]Technical University of Mombasa, Msambweni, Kenya, [10]Stanford University, Portola Valley, CA, United States

As dengue, chikungunya, and Zika transmission accelerates under urbanization and climate change, scalable tools for Aedes aegypti habitat surveillance are urgently needed. Satellite imagery lacks resolution to detect household-level breeding sites, while ground surveys are too labor-intensive to scale. Unmanned aerial vehicle (UAV) imaging bridges this gap, yet no study has validated whether different operators can consistently identify, delineate, and classify trash- a dominant breeding substrate- from aerial images. We conducted a multi-dimensional validation study across two epidemiologically distinct urban Kenyan sites (Ukunda, coastal; Kisumu, western) where arboviral transmission is endemic. High-resolution orthomosaic maps (3 cm/pixel) were generated from UAV flights covering 3.5 km². Trash areas were digitized in QGIS and classified using a 7-level scheme stratified by Ae. aegypti breeding risk, collapsed into three groups: tires, high-risk, and low-risk trash. Inter-rater reliability (three raters, 500 objects) and intra-rater reliability were assessed using Cohen's Kappa, Fleiss' Kappa, Krippendorff's Alpha, and spatial overlap metrics. Classification reliability was strong: average pairwise Cohen's Kappa was 0.80 (95% CI: 0.75-0.86), Fleiss' Kappa reached 0.90 by risk level (95% CI: 0.87-0.93), and fuzzy Krippendorff's Alpha was 0.94 (95% CI: 0.91-0.96), confirming that rare disagreements involved adjacent categories rather than meaningful misclassification. Spatial analysis showed consistent identification of major trash features, with variability concentrated at diffuse boundaries. Intra-rater consistency was high for well-defined features (4.7% unique area) and moderate for complex accumulations (18.5%). Across both sites, 3,204 trash piles were mapped. This validated methodology enables quantifiable comparisons of vector breeding risk across sites and over time and provides labeled training data essential for deep learning models toward automated vector surveillance at scale.

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RISK MAPPING AND DETERMINANTS OF THE POTENTIAL PRESENCE OF *ANOPHELES STEPHENSI* IN BENIN: A GEOSTATISTICAL AND LOGISTIC REGRESSION APPROACH

André Sominahouin

CREC/BENIN, Cotonou, Benin

Anopheles stephensi is an emerging malaria vector, historically confined to Asia but recently reported in several African countries. Its ability to adapt to urban environments represents a growing threat to vector control strategies. In Benin, the presence of environmental conditions conducive to its establishment suggests a potential risk of occurrence, although this has not yet been confirmed by comprehensive entomological data. This study aimed to identify determinants associated with the risk of potential presence of *Anopheles stephensi* and to map its probable spatial distribution at the national level. An ecological study covering the 77 municipalities of Benin was conducted using agricultural, livestock, socioeconomic, demographic, and environmental data collected between March and May 2025. The dependent variable did not reflect observed vector presence but rather an indicator of the ecological suitability of larval habitats to support *Anopheles stephensi*. Multivariable logistic regression, followed by probabilistic spatial modeling, was used to estimate the likelihood of potential vector presence. Human population density ($p = 0.001$) and swine population size ($p < 0.001$) were positively associated with the risk of potential presence of *Anopheles stephensi*, whereas cattle, sheep, and goat populations showed significant negative associations. The maps produced represent probabilities of occurrence rather than confirmation of actual vector presence. In conclusion, this study highlights areas of Benin with a high probability of potential presence of *Anopheles stephensi*. These findings provide decision-support tools to guide prioritized entomological surveillance and preventive interventions before confirmed establishment of the vector.

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LIFE-HISTORY DIVERGENCE IN *AEDES AEGYPTI* (DIPTERA: CULICIDAE) ALIGNS WITH KENYA'S DENGUE LANDSCAPE

Jed Igwe

Texas Tech University, Lubbock, TX, United States

Dengue outbreaks in Kenya are concentrated along the coast despite widespread ecological suitability for *Aedes aegypti* inland, suggesting that transmission risk is shaped by more than climate alone. Population-level variation in mosquito life-history traits—interacting with vector competence, climate-driven abundance, and socio-ecological factors—may contribute to this heterogeneity, yet direct comparative data of differences in life history traits across Kenyan populations remain scarce.Using a common-garden experiment with minimally colonized populations, we compared early survival, developmental timing, and short-term adult survival among *Ae. aegypti* from coastal Mombasa, inland Kisumu, and a laboratory-adapted Mexico colony under identical insectary conditions. Substantial colony-level differences persisted: Mombasa mosquitoes showed higher early-stage success and faster development, Kisumu mosquitoes exhibited reduced early performance and slower development, and the Mexico colony maintained consistently high survival. Although derived from single field collections, these findings demonstrate that meaningful variation in key life-history traits can persist among Ae. aegypti colonies when evaluated under standardized conditions. Such differences may influence local population growth dynamics, but do not alone determine transmission risk. Incorporating population-specific mosquito biology into surveillance and modeling frameworks—alongside measures of vector competence, adult behavior, and human exposure—may improve spatial risk prediction and support more context-appropriate vector control strategies.

7065

SURVEILLANCE OF LIVESTOCK DISEASE VECTORS IN KOREA - DISTRIBUTION AND VIRAL DETECTION IN 2025

Eun-Ju Kim, Jihun Ryu, Jeong-Uk Jeong, Youjin Han, Hye-Young Jeoung, Hae-Eun Kang, SeEun Choe

Animal and Plant Quarantine Agency, Gimcheon-si, Gyeongbuk, Republic of Korea

Climate change and global trade increase the risk of transboundary livestock disease vectors entering Korea, highlighting the need for continuous surveillance. Mosquitoes, biting midges (*Culicoides* spp.), and stable flies (*Stomoxys calcitrans*) are important vectors transmitting diseases such as Japanese encephalitis, West Nile fever, Bluetongue, and Lumpy skin disease. This study aimed to investigate the distribution and viral pathogens of major livestock disease vectors at 120 cattle farms in Gangwon, Gyeonggi, Chungnam, and Jeonbuk provinces. From May to November in 2025, vectors were collected monthly using black light traps and sticky traps, and identified through morphological and genetic methods. Collected specimens were pooled by species and sampling site, and viral pathogens detection was performed using real-time RT-PCR assays targeting major livestock viruses. Trap index (TI) was calculated as the mean number of insects collected per trap per night. A total of 27,346 mosquitoes, 10,048 biting midges, and 3,282 stable flies were collected. The dominant mosquito species was *Aedes vexans* (40.6%), followed by *Anopheles* spp. (30%) and *Culex pipiens* (13.6%). Among biting midges, *Culicoides arakawae* was dominant (70.7%). Mosquito abundance peaked in July-August, while biting midges were most abundant from August to October. Stable flies were collected evenly through the sampling period. The TI values for mosquitoes varied by region, ranging from 15.8 to 43.2. TI values for biting midges ranges from 14.7 to 35.0 while those for stable flies ranged from 1.9 to 5.4. Pathogen screening detected Japanese encephalitis virus genotype 1 in one pool of *Culex pipens* from Jeonbuk, while all other tested pathogens were negative. These results indicate distinct seasonal patterns among vector groups and confirm the presence of arboviruses in livestock-associated vectors. This study provides baseline data for early detection of emerging livestock and supports ongoing vector surveillance in Korea.

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MALARIA VECTORS DIVERSITY, SEASONALITY IN SUSTAINING DISEASE TRANSMISSION IN AN ENDEMIC AREA OF KATI-FALADIE, MALI

Fatalmoudou Tandina, Safiatou Niare Doumbo, Moussa Djimde, Sekou Sissoko, Privat Agniwo, Mathias Dolo, Abdramane Kamate, Amatigue Zeguime, Abdoulaye Djimde, Laurent Dembele

Parasites & Microbes, research and training center, Bamako, Mali

Anopheles coluzzii, An. gambiae(s.s.), An. arabiensis and *An. funestus* mosquitoes are the dominant vector species of *Plasmodium* parasites transmission in Mali. The vector massive circulation is observed during the rainy season and clearly decreases during dry season with only a few mosquitoes able to survive during this season. The main objective is to assess the diversity of Anopheles mosquito populations during dry and rainy seasons. Furthermore, this study aimed to identify the major species sustaining continued malaria transmission across seasons. From a cross-sectional study in malarias endemic area, mosquitoes were captured twice, the rainy and dry seasons using pyrethroid spray catches in Kati-Faladie. Collected mosquitoes were identified according to the morphological keys and molecular techniques such as PCR-RFLP (IGS regions). *Plasmodium spp* positivity in each mosquito was characterized by qPCR using parasite 18 S genes**.** In Kati-Faladie site, 1,082 and 1,705 mosquitoes were captured in rainy and dry seasons respectively inside the same houses. In rainy season 454 (42%) were anophelines and 624 (58%) culicines. During dry season, 65 (3.81%) of the captured mosquitoes were *Anopheles*, while 1,640 (96.19%) were *Culex.* Morphologically, anopheline population consisted of 100% *An. gambiae* s.l. *Anopheles* species diversity in rainy season was comprised of the sister taxa, *An. gambiae s.s* and *An. coluzzii* followed by *An. gambiae/An. coluzzii* hybrid and *An. arabiensis*. However, only *An. coluzzii* was found as vector across rainy and dry seasons sustaining malaria transmission. *Anopheles* infection rate of *Plasmodium falciparum* was 10.5% and 3.7% for rainy and dry respectively. *Anopheles coluzzii* species can survive in dry season and could sustain malaria transmission. This highlights the importance of tackling *Anopheles coluzzii* species and residual malaria transmission to eradicate the disease in rural area of Mali.

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URBAN-SCALE APPLICATION OF LONG-LASTING REPELLENT TEXTILES: TRANSLATIONAL LESSONS FROM FIELD DEPLOYMENT

Álvaro Eiras

Innovec LTDA, Belo Horizonte, Brazil

Urban mosquito control requires complementary tools that are effective, durable, and acceptable under real-world conditions. Long-lasting spatial repellent textiles represent a promising approach; however, translating efficacy from laboratory and semi-field studies into scalable urban products remains a challenge.This study aimed to provide data-driven guidance for translating experimental findings into industrially scalable repellent textiles for urban use. Evidence was synthesized from laboratory assays, semi-field evaluations, and urban and peri-urban field deployment in Brazil to identify constraints affecting performance beyond controlled settings.Laboratory and semi-field studies demonstrated consistent spatial repellency against *Aedes aegypti* and *Anopheles darlingi*, influenced by textile architecture, emission surface, airflow, and placement. Based on these findings, 400 repellent-treated textile footwear units were deployed under routine conditions to assess durability, operational performance, user compliance, and perceived effectiveness.Field results showed that reductions in mosquito biting pressure can be achieved when textile configuration and emission surfaces are optimized. User feedback indicated high acceptability and sustained use, emphasizing the importance of comfort, aesthetics, and perceived protection. Key challenges included balancing release rates with durability, maintaining efficacy after washing and wear, and ensuring consistent performance under variable environmental conditions. These findings demonstrate how applied entomology, material design, and user-centered deployment can be integrated to support scalable spatial repellent textiles for urban mosquito control.

7068

PHASING OUT OF DDT FROM INDOOR RESIDUAL SPRAYING (IRS), OPERATIONS FOR MALARIA CONTROL IN LIMPOPO PROVINCE, SOUTH AFRICA

Gladstone Thabo Madito

University of Cape Town, Cape Town, South Africa

Malaria remains a major public health concern in the Limpopo Province, South Africa, despite substantial reductions in transmission achieved over the past two decades. These improvements have been largely driven by the widespread use of indoor residual spraying (IRS), particularly with dichlorodiphenyltrichloroethane (DDT), which has been effective in reducing Anopheles vector populations and interrupting local transmission dynamics. Concerns regarding the environmental impacts of DDT have prompted renewed interest in next-generation insecticides and integrated vector control strategies. In addition, extreme weather events, including heatwaves and flooding, have been associated with surges in mosquito populations and localized malaria outbreaks, even in areas with high IRS coverage in the province. These conditions affect vector survival and malaria seasonality and may compromise existing control measures and the effectiveness of IRS activities. Several modelling studies emphasize the importance of including climatic drivers, insecticide selection, as well as the timing and coverage of spray operations into malaria transmission models. While next-generation insecticides have demonstrated promising residual activity in controlled trials, there remains a need to evaluate their real-world effectiveness on malaria incidence in heterogeneous settings,

particularly when supplemented with larviciding. Understanding these factors enables malaria control programmes to evaluate phased strategies to inform the design of cost-effective integrated control strategies. We developed a spatially explicit malaria transmission model for Limpopo province at multiple administrative levels to evaluate the cost-effectiveness of DDT replacement strategies. We assess the potential of next-generation insecticides and integrated control packages to sustain and accelerate progress toward malaria elimination. Our findings aim to support operational planning for IRS scale-down and resource allocation to guide cost-effective withdrawal of DDT from IRS activities.

7069

AN ETHICAL AND ACCESSIBLE APPROACH TO ITN TESTING: THE HOST-FREE TUNNEL TEST

Kath Gleave[1], Jessica Williams[2], Hannah Dobbins[2], Derric D. Nimmo[1]

[1]IVCC, Liverpool, United Kingdom, [2]LITE, Liverpool, United Kingdom

The tunnel test is a laboratory bioassay recommended by the WHO as a standard method for 'free-flying' testing of ITN bioefficacy. Data generated using the tunnel test are routinely used by ITN manufacturers in WHO Prequalification dossier submissions to demonstrate efficacy, and by implementers and country teams to monitor the durability of performance of ITNs over time. The tunnel test consists of two chambers in which mosquitoes must pass through an ITN sample containing 9 standard holes (1cm) to reach a live animal bait. The bait provides cues (odour, CO_2 and heat) that attract female mosquitoes, prompting them to contact the ITN sample. Mosquitoes are then scored for passage rate, blood-feeding and mortality following exposure. Although recognised by the WHO and widely used in ITN testing facilities, the method has limitations. The need for an animal host requires an animal handling licence, ethical approval and ongoing maintenance costs. These restrictions limit accessibility and even where facilities can conduct this testing, the ethical desire to find an alternative method which removes the reliance on animal use is commonly voiced. To address these issues, a host-free tunnel test methodology has been developed between LITE and IVCC. Baseline data, testing with untreated netting, confirm the method is appropriate, with over 50% blood feeding and less than 10% mortality. Validation for use with novel chemistries and dual-active ingredient (AI) products is underway. This development represents an important advancement in vector control product testing, as it provides an ethically responsible, cost-effective and scalable alternative to animal-dependent assays. By removing the need for live-hosts, the host-free tunnel test will allow widespread adoption of testing and can enhance standardisation across laboratories, improving consistency and comparability of results. This innovation has the potential to accelerate product development and strengthen the effects to get new tools to market to combat insecticide resistance and sustain effect malaria control.

7070

EVALUATING NOVEL MULTI-VECTOR CONTROL TOOLS FOR HUMANITARIAN CRISES: THE ENTOMOLOGICAL EFFICACY OF LONG-LASTING LARVICIDE IN NORTHERN NIGERIA

Richard James Allan[1], Mohammad Kamal[1], Umar B. Goni[2], Blaise N. Alenwi[2], Sajjad Ahmad[2], Sara Estecha-Querol[1], Garba M. Ashir[3], Godwin Nwakanma Ntadom[4], David Weetman[5], Louisa Alexandra Messenger[6]

[1]The MENTOR Initiative, Haywards Heath, United Kingdom, [2]The MENTOR Initiative, Maiduguri, Nigeria, [3]The Univeristy of Maiduguri, Maiduguri, Nigeria, [4]Federal Ministry Of Health, Nigeria, FCT, Nigeria, [5]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [6]University of Nevada, Las Vegas, Henderson, NV, United States

By mid-2025, 117.3 million people globally had been forcibly displaced from their homes due to persecution, conflict, violence, or human rights violations. In emergency settings, displaced populations, residing in flimsy shelters and experiencing food insecurity, inadequate sanitation and poor access to health services, are especially susceptible to vector-borne diseases (VBDs). Conventional vector control interventions are often operationally and biologically unfeasible to protect such populations from VBDs. We conducted a 6-month, 2-arm community trial in 343 shelters in two internally displaced people camps in the protracted humanitarian crisis in Borno State, Nigeria, to evaluate the entomological efficacy of SumiLarv^{TM} 2MR, a long-lasting larvicide for use in water containers, against major malaria and dengue vector species. Monthly entomological monitoring measured changes in household immature and adult vector density. Intervention acceptability was assessed using focus group discussions and a cross-sectional survey. Baseline household vector positivity was higher in the intervention arm than the control (PR: 2.32 [95% CI: 1.40 - 3.85]; p=0.001). In the control arm, household immature-stage positivity declined significantly from the baseline to post-intervention period (PR: 0.57 [95% CI: 0.33 - 0.98]; p=0.041). After accounting for temporality, the intervention-by-period interaction demonstrated a significantly greater reduction in household immature-stage positivity in the intervention arm relative to the control (DID PR: 0.49 [95% CI: 0.25 - 0.94]; p=0.033). Similarly, a significant reduction in household positivity for adult *Ae. aegypti* (β: -0.22 [95% CI: -0.34 - -0.093]; p=0.001) and overall adult *An. gambiae* s.l. density (DID IRR: 0.096 [95% CI: 0.065 - 0.141]; p<0.001) was observed. The intervention was well received by participants, with 99.1% reporting SumiLarv 2MR was easy to use, 99.7% being willing to use it again and 99.4% being willing to pay for SumiLarv 2MR. Long-lasting larvicide has the potential to be integrated into the expanding toolbox to protect the forced displaced from multiple VBDs.

7071

OPERATIONAL OUTCOMES AGAINST *AEDES AEGYPTI* DURING A PAST ZIKA OUTBREAK IN JAMAICA

Carolina Torres Gutierrez[1], Sashell Mackenzie[2], Kimalie Parchment[3], Jason Brinkley[4], Marci Schalk[5], Sherine Huntley Jones[6], Kerri-Ann Guyah[5], Jean Margaritis[7], Allison Belemvire[8], Sheena Francis[9]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Minister of Agriculture, Fisheries, and Mining, Kingston, Jamaica, [3]Caribbean Institute for Health Research, University of the West Indies, Mona, Kingston, Jamaica, [4]Associate Professor of Biostatistics, Office of Clinical Research Statistics, Research Intelligence, Northwell Health, New York., New York, NY, United States, [5]Abt Global, Rockville, MD, United States, [6]Ministry of Health and Wellness of Jamaica., Kingston, Jamaica, [7]Peace Corps Malawi, Lilongwe, Malawi, [8]U.S. Department of State, Global Health Security and Diplomacy, Washington D.C., MD, United States, [9]Natural Products Institute, University of the West Indies, Mona, Kingston, Jamaica

The Zika AIRS Project (ZAP), a USAID-funded initiative, worked across Latin America and the Caribbean as an emergency response supporting Ministries of Health to reduce the burden of the Zika virus during the 2015-2018 outbreak. The implementation in Jamaica involved monitoring of the mosquito vectors as well as a wide scale biolarvicide application as a control strategy. *Aedes aegypti* is known as the Zika, dengue and chikungunya vector. In Jamaica, we assessed three *Ae. aegypti* trapping methodologies to evaluate the efficacy of a wide range biolarvicide application in domestic environments. Traps used were BG-sentinel, Gravid (GAT) and Prokopack aspirators (PK). Trapping devices were deployed in four areas, three of which were treated with a biolarvicide (VectoBac WDG), and designated as intervention sites. A designated control site was an area without biolarvicide treatment. Traps were deployed for a three-day period in four to eight houses. Each household had two sampling periods/month. Statistical analyses followed zero-inflated negative binomial regression, and models for mosquito count data were used to explore differences between intervention and control sites, adjusting for trap type and date. Our efforts produced 7349 mosquitoes trapped by all devices, with 4674 *Ae. aegypti* individuals (63%). Overall, the number of *Ae. aegypti* females per trap per person per house was higher in the control site. On average, the number of *Ae. aegypti* females trapped by PK was 10.8 females in the control site, compared to 5.44 in the intervention site. Comparatively, for BGs and GATs, the average number of females per collection period was

5.89 and 2.27 respectively in the control site, and 2.45 and 0.87 females per collection in the intervention sites. There was statistical difference for trap and site covariates (p-values less than 0.05). Our findings showed that PK aspirators though less specific to *Ae. aegypti* were operationally friendly, efficient and widely accepted by local communities. ZAP's mosquito control implementation in Jamaica through a larvicide campaign achieved a reduction of 31.5% in female *Aedes* across the intervention sites.

7072

EARLY FINDINGS ON TRANSFLUTHRIN-BASED PASSIVE EMANATORS FOR IN-HOME *AEDES AEGYPTI* CONTROL IN PONCE, PUERTO RICO

Chelsea G. Major[1], Nexilianne Borrero-Segarra[2], Olga D. Lorenzi[1], Marjorie Y. Martínez[3], Fhallon C. Ware-Gilmore[4], Raiza Alvarado Figueroa[5], Dania M. Rodríguez[1], Grayson C. Brown[4], Ryan R. Hemme[1], Gabriela Paz-Bailey[1], Julieanne Miranda-Bermúdez[4], Vanessa Rivera-Amill[3], Laura E. Adams[1]

[1]Centers for Disease Control and Prevention, San Juan, PR, United States, [2]Puerto Rico Vector Control Unit, Ponce, PR, United States, [3]Ponce Health Sciences University/Ponce Research Institute, Ponce, PR, United States, [4]Puerto Rico Vector Control Unit, Puerto Rico Science Technology and Research Trust, San Juan, PR, United States, [5]Puerto Rico Science Trust, Aguirre, PR, United States

Passive emanators (PEs), also known as spatial repellants, are novel devices that disperse the synthetic pyrethroid transfluthrin to repel and disorient mosquitoes, reducing their ability to locate and bite hosts. PEs may offer an effective in-home approach for controlling mosquitoes, including *Aedes aegypti*, the primary vector for dengue virus (DENV). To assess its effectiveness in reducing *Ae. aegypti* and DENV transmission, we are evaluating the SC Johnson Guardian™ PE in the Communities Organized to Prevent Arboviruses (COPA) cohort in Ponce, Puerto Rico. Homes with COPA participants in 38 communities were enrolled and randomized 1:1 to intervention or control. Visits occur at 0, 3, 6, 9, and 12 months and include household questionnaires and serum collection for DENV testing; 30% of homes also receive monthly indoor mosquito aspirations. At 0-mo visits, study staff installed PEs on interior walls of intervention homes based on room volume per manufacturer guidelines. Here we present early findings. From Oct 2024-Jul 2025, 903 homes were enrolled; 429 (48%) were intervention homes with a mean 11.6 PEs per home. In 3-mo visits (821 homes), 49% (205/416) of intervention vs. 21% (85/405) of control homes reported indoor mosquitoes had decreased (χ^2 $P < 0.001$). Similarly, 50% vs. 20% reported a decrease in mosquito bites (χ^2 $P < 0.001$). Most intervention homes (378/416, 91%) reported satisfaction with PEs; among 18 unsatisfied, the most common reason was lack of mosquito reduction (12/18, 67%). In 203 homes with aspirations, mean female *Ae. aegypti* at months 0, 3, and 6 was 1.0, 0.56, and 0.38 in intervention homes and 0.89, 0.72, and 0.84 in control homes ($P = 0.025$ for intervention vs. control at 6 months). The proportion of homes with ≥1 *Ae. aegypti* female was 28%, 20%, and 21% in intervention and 27%, 24%, and 26% in control homes. These results suggest that PE devices had high acceptability and produced some perceptible reductions in mosquito bites and female *Ae. aegypti* in the 6 months post-installation. Findings will be updated based on ongoing data collection.

7073

A ONE HEALTH APPROACH TO VECTOR CONTROL: ASSESSING THE MOSQUITOCIDAL POTENTIAL OF ENDECTOCIDES AGAINST *ANOPHELES GAMBIAE* S. L

Cornelia Appiah-Kwarteng[1], Yaw Akuamoah-Boateng[1], Isaac Sraku[1], RIchard T. Doe[1], Yaw A. Afrane[2]

[1]University of Ghana, Accra, Ghana, [2]University Of Ghana, Accra, Ghana

Insecticide resistance and outdoor biting behaviour have eroded the effectiveness of conventional malaria vector control tools, prompting interest in endectocides. While ivermectin is the most studied endectocide for mosquito control, there are reports of resistance, and thus new candidates are imperative. The mosquitocidal and anti-fecundity effects of piperazine, albendazole and ivermectin were evaluated against *Anopheles gambiae* mosquitoes, using membrane and direct feeding assays; test controls were mosquitoes fed with no endectocides. The number of eggs laid and surviving mosquitoes were recorded daily for 21 days post-feeding to determine fecundity, reproductive rate, intrinsic growth rate and survivorship. In membrane feeding assays, albendazole-spiked blood fed mosquitoes (0.49±0.19 χ^2=11.36, p<0.001) and piperazine-spiked blood fed mosquitoes (0.54±0.07 χ^2=20.30; p<0.001) showed lower survivorship, compared to the control (0.70±0.27). Piperazine entirely suppresed fecundity, reproductive rate and growth rate of mosquitoes compared to albendazole (0.59; 0.37; 0.40) and the control (0.80, 0.69, and 0.55 respectively). In direct feeding assays, survivorship was lowest in ivermectin- and piperazine-spiked blood fed mosquitoes (0.36±0.10 and 0.57±0.22), compared to albendazole- and the non-spiked blood fed controls (0.75±0.12 and 0.83±0.06). Fecundity and reproduction were suppressed in piperazine-spiked blood fed mosquitoes (0.01), comparable to ivermectin-spiked blood fed mosquitoes (0.00). Albendazole showed no significant impact on egg production (0.47) or offspring viability (0.38) compared to controls (0.40 and 0.38). Growth rates were highest in control groups (0.40), moderately reduced with albendazole exposure (0.26), and turned negative with piperazine treatment (-0.08), indicating the potential for mosquito population decline under piperazine exposure. These findings support the use of piperazine in the control of malaria vectors, and also the exploration of a broader endectocide toolkit, within integrated One Health approaches to malaria vector control.

7074

BRIDGING TRADITIONAL MEDICINE AND VECTOR BIOLOGY: MODULATION OF MOSQUITO MICROBIOTA AND *PLASMODIUM FALCIPARUM* DEVELOPMENT BY PLANT EXTRACTS

Aminata Fofana, Fabrice Anyirekun Some

Institut de Recherche en Sciences de la Santé, Bobo-Dioulasso, Burkina Faso

Malaria remains a major public health challenge in Africa, particularly in Burkina Faso, where *Plasmodium falciparum* is the predominant parasite. In the context of increasing resistance of both the parasite and its vector (*Anopheles gambiae* complex), medicinal plants represent a promising alternative, although their effects on vector biology remain poorly understood. This study evaluated the effects of three traditional medicinal plants (*Sida alba*, *Guiera senegalensis*, and *Sarcocephalus latifolius*) on parasite development and mosquito fitness. Extracts were obtained by dichloromethane maceration and characterized by quantifying secondary metabolites. Experimental infections were performed using membrane feeding assays with two concentrations (0.025 and 0.25 mg/ml). Oocyst prevalence and intensity were measured, along with mosquito survival and fecundity. Phytochemical analysis showed significant variability among plants, with *G. senegalensis* exhibiting the highest metabolite levels ($p < 0.0001$). No significant reduction in infection prevalence or intensity was observed. However, a marked increase in oocyst load was detected in *An. coluzzii* exposed to *Sida alba* (38.3 vs. 1.7 in controls). Additionally, *G. senegalensis* and *S. latifolius* significantly increased mosquito survival (5 vs. 3 days) and fecundity (50% vs. 11.1%). These findings suggest that, at the tested doses, plant extracts may enhance mosquito fitness and favor parasite development. Further studies are needed to assess dose-dependent effects and implications for malaria transmission.

7075

SEMI-FIELD VALIDATION OF STERILE INSECT TECHNIQUE EFFICACY AND POPULATION STUDIES IN *AEDES AEGYPTI*

Benjamin L. Nyman[1], Solomon Kibret Birhanie[2], Michelle Brown[3], Karthikeyan Chandrasegaran[1]

[1]University of California Riverside, Riverside, CA, United States, [2]West Valley Mosquito and Vector Control District, Ontario, CA, United States, [3]West Valley Mosquito and Vector Control Distsrict, Ontario, CA, United States

Integrated vector management depends on accurately assessing mosquito threat and deploying cost-effective, sustainable control. Sterile insect technique (SIT) meets this need by reducing vector populations without insecticides, as wild females mate with sterilized males that produce no viable offspring. Yet optimizing SIT programs for invasive *Aedes* requires better understanding of how sterilization methods, production, and release strategies perform outside the lab. Most current parameters come from cage studies, where fitness costs of sterilization such as reduced activity or competitiveness are difficult to detect. SIT programs also rely on trap data to guide release ratios derived from these trials, though how well traps reflect local populations remains unclear. We addressed these gaps using a semi-field system: an 8'×18'×15' greenhouse filled with field-derived *Ae. aegypti* females alongside males that were either untreated or irradiated at 55 Gy in varying ratios (1:0, 0:1, 1:1, 1:5). After 72 hours, BG-S traps were deployed daily to recapture mosquitoes alive. Females were then blood-fed and isolated for oviposition to measure fecundity. Subgroups of both sexes were marked with an acrylic-bound UV fluorophore delivered via nebulizer to test effects on recapture and reproduction, and to identify sterile males. In this semi-field setting, the proportion of females producing no viable eggs closely tracked sterile male release ratios. Marking was highly efficient and had no detectable effect on recapture or reproduction in either sex, though males were consistently underrepresented in trap collections. In combination, this study advances SIT research and operational programs by piloting methods and analyses that bolster how we evaluate vector populations and how we combat them. These results validate lab-derived release parameters under more realistic conditions and highlight the value of assessing recaptured females in semi-field contexts. The marking approach also offers a practical tool for generating operational data critical to SIT-based vector management.

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MACHINE LEARNING IMPROVES SYNDROMIC DENGUE DETECTION AND CASE DEFINITION PERFORMANCE IN EL SALVADOR

Zachary J. Madewell[1], Stephanie Bellman[2], Jose Elias Aguilar Ramirez[3], Kaci D. McCoy[2], Parminder Suchdev[4], Kenni Campos[5], Kathleen Weimer[2], Delmy Lisseth Recinos[3], Rafael Chacón-Fuentes[4], Emily Zielinski-Gutierrez[4], Xochitl Sandoval[3], Shannon E. Ronca[6], Laura E. Adams[1], Gabriela Paz-Bailey[1], Rhina Dominguez[3], Kristy O. Murray[2]

[1]Division of Vector-Borne Diseases, CDC, San Juan, PR, USA, San Juan, PR, United States, [2]Emory School of Medicine, Atlanta, GA, United States, [3]National Institute of Health of El Salvador, San Salvador, El Salvador, [4]Centers for Disease Control and Prevention, Central America Region, Guatemala City, Guatemala, [5]Pediatra Centro Médico de Santa Ana, Santa Ana, El Salvador, [6]Baylor College of Medicine, Houston, TX, United States

Distinguishing dengue from other acute febrile illnesses (AFIs) is difficult because symptoms are non-specific and overlap with other febrile syndromes, particularly where diagnostic testing is limited. We assessed whether Extreme Gradient Boosting (XGBoost) could identify clinically interpretable predictors of reverse-transcription polymerase chain reaction (RT-PCR)-confirmed dengue and inform refinement of symptom-based dengue case definitions in El Salvador. Using prospective AFI surveillance data from Santa Ana from January 2022 through July 2024, we analyzed 5,068 patients, including 641 laboratory-confirmed dengue cases. We trained and validated XGBoost models using demographic, symptom, and comorbidity data with a 70/30 train-test split and 5-fold cross-validation. Performance was compared with clinician suspicion and the 2023 and 2024 national probable dengue case definitions. XGBoost accurately distinguished RT-PCR-confirmed dengue from other AFIs (AUC 0.94, 95% CI 0.92-0.96). Strong predictors were absence of respiratory symptoms—especially cough, shortness of breath, sneezing, sore throat, and wheezing—plus flushing, headache, and younger age. The highest-performing symptom combination (absence of shortness of breath, sneezing, sore throat, and wheezing) yielded 92.0% sensitivity, 73.5% specificity, and 98.5% negative predictive value. Clinician suspicion showed 80.5% sensitivity and 87.0% specificity. The 2023 case definition showed 58.8% sensitivity and 80.5% specificity, while the stricter 2024 definition had lower sensitivity (30.4%) but higher specificity (98.1%). Adding absence of cough to the 2023 definition increased specificity to 93.7%, with modestly lower sensitivity (55.2%). Machine learning identified simple, clinically interpretable features that can enhance dengue case definitions without additional laboratory testing. Incorporating absence of respiratory symptoms may improve clinical triage, syndromic surveillance, and outbreak detection in resource-limited settings.

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PERFORMANCE COMPARISON OF TWO DENGUE NS1 RAPID DIAGNOSTIC TESTS AGAINST RT-PCR

Chi-chun Tsai[1], Po-Chih Chen[2], Ching-Yi Tsai[3]

[1]Division of Infectious Diseases, Tropical Medicine Center, Kaohsiung Medical University Hospital, Kaohsiung City, Taiwan, [2]Department of Laboratory Medicine, Kaohsiung Medical University Hospital, Kaohsiung City, Taiwan, [3]Tropical Medicine Center, Kaohsiung Medical University Hospital, Kaohsiung City, Taiwan

Dengue virus (DENV) is a mosquito-borne flavivirus that remains a global public health threat, particularly in tropical regions where it is endemic. Accurate and timely diagnosis is critical for optimal patient care and effective outbreak containment. While reverse transcription-polymerase chain reaction(RT-PCR) remains the gold standard for dengue diagnosis, nonstructural protein 1 (NS1) rapid diagnostic tests (RDTs) offer a more accessible alternative. However, their performance may vary with infection type, serotype, and the timing of sample collection. We evaluated the diagnostic performance of two NS1 RDTs, Abbott NS1 and PBF NS1, against RTPCR as the reference standard. A total of 183 dengue-positive and 40 dengue-negative samples were obtained during the 2023 dengue outbreak in Kaohsiung, Taiwan. Sensitivity, specificity, and predictive values were comprehensively assessed based on infection type, serotype, and post-symptom onset (PSO) intervals. Additionally, we analyzed the diagnostic benefits of combining NS1 detection with anti-DENV IgM serology. Abbott NS1 exhibited notably higher sensitivity (87.4 % vs. 83.1 %) and specificity (100 % vs. 97.5 %) compared to PBF (Panion & BF Biotech Inc., Taiwan) NS1, though the differences were not statistically significant. Sensitivity varied by PSO, with Abbott NS1 achieving significantly higher sensitivity beyond three days (97.4 % vs. 76.9 %, P = 0.034). In secondary infections, Abbott NS1 outperformed PBF NS1 (86.8 % vs. 79.2 %). When combined with anti-DENV IgM serology, both RDTs demonstrated improved diagnostic sensitivity, particularly in secondary infections. Our findings underscore the importance of context-specific diagnostic tools. Combining NS1 antigen detection with IgM serology enhances diagnostic accuracy. While Abbott NS1 demonstrated slightly better performance, PBF NS1 remains a viable and non-inferior alternative.

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EPIDEMIOLOGY AND CLINICAL CHARACTERISTICS OF DENGUE AND CIRCULATING SEROTYPES DETECTED THROUGH THE EL SALVADOR ACUTE FEBRILE ILLNESS NETWORK, 2022-2024

Stephanie Bellman[1], Zachary Madewell[2], José Elías Aguilar Ramírez[3], Kaci D. McCoy[1], Parminder Suchdev[1], Kenni Campos[3], JongIn Hwang[1], Kathleen Marie Weimer[1], Delmy Lisseth Recinos[3], Sarah M. Gunter[4], Rafael Chacon[5], Emily Zielinski-Gutierrez[6], Xochitl Sandoval[3], Shannon Ronca[4], Laura Adams[2], Gabriela Paz-Bailey[2], Rhina Dominguez[3], Kristy O. Murray[1]

[1]Emory University, Atlanta, GA, United States, [2]CDC, San Juan, PR, United States, [3]Instituto Nacional de Salud, San Salvador, El Salvador, [4]Baylor College of Medicine, Houston, TX, United States, [5]Univ Del Valle de Guatemala, Guatemala, Guatemala, [6]CDC/DVBID, Fort Collins, CO, United States

Dengue is a common vector-borne disease in the Americas, yet diagnosis remains challenging because symptoms overlap with other acute febrile illnesses and clinical recognition may vary by the circulating serotype (Dengue virus (DENV) 1-4). The El Salvador Acute Febrile Illness Network was established to characterize the infectious causes of fever and prospectively enrolled participants of all ages through facility-based surveillance from January 2022-July 2024. Participant clinical signs/symptoms and demographic data were recorded, followed by serotype-specific qPCR testing for dengue virus (DENV) for all patients with available samples. Descriptive statistics and logistic regression were performed to assess differences in clinical presentation by serotype. Among 5,607 participants tested, 641 were positive (11.4%) for DENV, with 66 (10.3%) DENV-1, 5 (0.8%) DENV-2, 460 (71.8%) DENV-3, and 110 (17.2%) DENV-4 infections. Most cases (71%) occurred during a DENV-3 outbreak beginning in late 2023. While respiratory symptoms were most common among participants without dengue, dengue patients most frequently had redness/flushing (72.4%), headache (69.0%), and chills (52.9%). Clinical presentations varied by serotype. Compared with DENV-4, DENV-3 cases more often had classic dengue features, including flushing (82% vs 34%), retro-orbital pain (43% vs 15%), and ≥2 gastrointestinal symptoms (47% vs 7%), and were more often clinically suspected as dengue (90% vs 40%), hospitalized (73% vs 51%), and classified as severe/grave dengue (6% vs 1%). In contrast, DENV-4 cases more often had respiratory features such as cough (25% vs 5%) and two or more respiratory symptoms (26% vs 5%), suggesting a more atypical presentation that may reduce clinical recognition. While dengue was the most common vector-borne disease detected, clinical presentations by serotype differed, creating difficulty in identification of DENV infections. Ongoing virological surveillance of circulating serotypes can improve clinician awareness and support more accurate and timely dengue diagnosis.

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DATA DRIVEN PREDICTIVE MODELING OF CASE FATALITY AND INCIDENCE RATES OF LASSA FEVER OUTBREAKS IN ONDO STATE, NIGERIA

Ayokunle O. Orimolade

Ondo State Miniistry Of Health, Akure, Nigeria

Lassa fever remains a major public health challenge in Nigeria, with Ondo State consistently recording some of the highest numbers of confirmed cases and deaths nationwide. Although surveillance and control measures are ongoing, limited application of predictive analytics has restricted early detection and timely response to outbreaks. This study aimed to develop and evaluate a data-driven predictive modeling framework for estimating and forecasting the case fatality and incidence rates of Lassa fever outbreaks in Ondo State, Nigeria. Retrospective epidemiological and surveillance data were obtained from the Ondo State Ministry of Health. The dataset included case classification, outcomes, reporting districts, health facility type, vaccination status, and data completeness indicators. Following data cleaning and exploratory analysis, predictive models—including multiple linear regression, random forest, and long short-term memory (LSTM) networks were developed to identify temporal and spatial patterns in outbreak dynamics. Model performance was assessed using the coefficient of determination (R^2), root mean square error (RMSE), and mean absolute error (MAE). Among the models evaluated, the LSTM network demonstrated superior predictive accuracy, achieving an R^2 of 0.93 and the lowest RMSE value (2.7). The overall case fatality rate among cases with known outcomes was 24.68%, while the recovery rate was 75.32%. Model forecasts projected a potential 12% increase in incidence during the early dry season (November-January) of 2026 if current control measures remain unchanged. Spatial mapping identified Owo, Akure North, and Ose districts as persistent hotspots, accounting for over 60% of predicted future cases. Machine learning-based predictive tools, particularly LSTM models, can enhance the accuracy of outbreak forecasting and strengthen Lassa fever surveillance systems. Integrating such data-driven approaches into public health programs could improve early warning systems, optimize resource allocation, and support evidence-based interventions in Ondo State and other Lassa fever-endemic regions of Nigeria.

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INCREASED RISK OF SAPOVIRUS INFECTION FOLLOWING NOROVIRUS GII-ASSOCIATED DIARRHEA IN A BIRTH COHORT IN PERU

Mayuko Saito[1], Macarema Danielli Vittet Mondonero[2], Robert H. Gilman[3], Neiza Vargas Claure[2], Holger Mayta[2], Gerardo Sanchez[2], Yusuke Sayama[1], Caryn Bern[4], Monica Jehnny Pajuelo[2]

[1]Tohoku University Graduate School of Medicine, Sendai, Japan, [2]Universidad Peruana Cayetano Heredia, Lima, Peru, [3]Johns Hopkins Univ Bloomberg School of Public Health, Baltimore, MD, United States, [4]University of California San Francisco, San Francisco, CA, United States

Sapovirus belongs to the *Caliciviridae* family, the same family as norovirus. Both viruses infect the small intestine and are frequently reported in community-based studies, causing a wide range of disease severity in childhood diarrhea across low- and high-income countries. However, interactions between these viruses remain poorly understood, particularly regarding how one infection may influence susceptibility to subsequent infections. We conducted a birth cohort study including 337 children in Peru from 2016 to 2019, with follow-up until two years of age. Stool samples were collected monthly and additionally during diarrheal episodes, and norovirus and sapovirus were detected using real-time PCR. The impact of norovirus-associated diarrhea on subsequent sapovirus infection was assessed by estimating the incidence of sapovirus-associated diarrhea within 30 days following norovirus genogroup II (GII)-associated diarrhea. Person-time data were analyzed using generalized estimating equations (GEE) and generalized linear models (GLM) with robust standard errors to account for within-individual correlation. Among 6,755 child-months of observation, 189 norovirus-associated diarrhea episodes and 182 sapovirus-associated diarrhea episodes were identified. The incidence of sapovirus-associated diarrhea was significantly higher within 30 days following norovirus GII-associated diarrhea compared with periods without such exposure (incidence rate ratio [IRR] = 3.7, 95% confidence interval [CI]: 2.4-5.7) after adjusting for age group. These findings were consistent across modeling approaches. The risk of sapovirus-associated diarrhea was significantly increased during the 30 days following norovirus GII-associated diarrhea, suggesting that norovirus-associated diarrhea may transiently increase susceptibility to subsequent sapovirus infection. This temporal association highlights a potential window of increased vulnerability following norovirus-associated diarrhea.

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DIFFERENCES IN ORAL HPV INCIDENCE AMONG A COHORT OF MEN RESIDING IN THE UNITED STATES AND BRAZIL STRATIFIED BY RACIAL AND ETHNIC IDENTITY WITH ASSOCIATED FACTORS

Christopher W. Dukes[1], Bradley Sirak[2], Anna R. Giuliano[2], Gillian H. Stresman[1]

[1]University of South Florida, Tampa, FL, United States, [2]Moffitt Cancer Center, Tampa, FL, United States

Over the last two decades, the incidence of human papillomavirus (HPV) associated oropharyngeal cancer (OPC) has been increasing, particularly among older and non-Hispanic white men, highlighting the need for better understanding of oral HPV infection natural history. This study investigates differences in incidence rates between racial and ethnic populations along with possible behavioral differences to identify future study and intervention targets. Data from men residing in the United States and Brazil enrolled in the HPV Infection in Men (HIM) Study oral subcohort were analyzed (n=2,116). Study participants self-reported racial and ethnic identity and were stratified into three groups for analyses including non-Hispanic White, non-Hispanic Black, and Hispanic. Participant characteristics and oral HPV incidence were presented with descriptive statistics. Factors associated with oral HPV incidence were assessed using multivariable adjusted time-varying Cox regression models (variables identified via backward selection) with 95% confidence intervals. Non-Hispanic Black men had consistently lower incidence rates of oral HPV infections compared to non-Hispanic White men, particularly for oncogenic HPV types (17.5% lower any oral HPV incidence; 34.5% lower oncogenic oral HPV incidence), while Hispanic men had the highest incidence of oncogenic oral HPV infections (3.2 per 1,000 person-months). Oral sex behavior was associated with oncogenic oral HPV incidence in every racial and ethnic population, with adjusted hazard ratios of 2.04 (95% CI = 1.35 - 3.09), 3.97 (95% CI = 1.73 - 9.10), and 2.79 (95% CI = 1.16 - 6.72) for non-Hispanic White, non-Hispanic Black, and Hispanic men who performed oral sex more than 6 times in the previous 6 months. In coclusion, our study shows that non-Hispanic White men have higher rates of oral HPV infections compared to non-Hispanic Black men; however, Hispanic men have the highest incidence of oncogenic infections. Performing oral sex is a significant risk factor in all populations.

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ANTIGENIC DIVERGENCE AMONG DENGUE VIRUS 1 LINEAGES MAY CONTRIBUTE TO PERSISTENCE IN BRAZIL

Michelle Premazzi Papa[1], Luke Trimmer-Smith[2], Michael A. Martin[1], Leah Katzelnick[3], Matthew H. Collins[4], Mauricio L. Nogueira[5], Derek Cummings[6]

[1]Johns Hopkins University, Bloomberg School of Public Health, Baltimore, MD, United States, [2]Gainesville, FL, United States, [3]NIAID/NIH, Bethesda, MD, United States, [4]Emory University, Division of Infectious Diseases, Atlanta, GA, United States, [5]Faculdade de Medicina de Sao Jose Do Rio Preto, SJ Rio Preto, Brazil, [6]Johns Hopkins University, Baltimore, MD, United States

The Dengue virus exists as four closely related but distinct serotypes (DENV 1-4). Infection with one serotype typically provides long-term homotypic immunity, but subsequent infection with a different serotype can lead to more severe disease due to antibody-dependent enhancement (ADE), in which prior immune responses facilitate viral entry into additional cells. Dengue is endemic in São José do Rio Preto (SJRP), Brazil, where DENV-1 L6 has circulated since 2008. In 2010, the DENV-1 L1 lineage was first detected, and both L1 and L6 co-circulated in the region. By 2013, L1 gradually declined until it was no longer detectable in the population. Analysis of DENV-1 epidemic dynamics and viral fitness showed that L1 had higher replicative efficiency than L6 in mosquitoes and in human and non-human primate cell lines. However, L6 infections exhibited reduced antigenicity, weaker B and T cell responses, and limited host immune interaction, leading to higher viremia. This enabled L6 to maintain greater epidemiological fitness and remain dominant despite the higher replicative fitness of L1.In this study, we characterized DENV-1 L1 and L6 lineages using an antigenic map generated from 20 African green monkey (AGM) sera infected with DENV 1-4 strains from Asia and the Americas. Nine of ten sera tested neutralized DENV-1 by focus reduction neutralization assay L1 (FRNT50: 22.64-1099.81), whereas only four of ten neutralized DENV-1 L6 (FRNT50: 51.16-1313.53). Among three sera from DENV-1 infected AGM, two sera showed similar neutralization responses, but one showed reduced neutralization of L6 (FRNT50 187 95% CI 108, 295) compared to L1 (FRNT50 334 95% CI 204, 560). We also analyzed neutralization titers of a contemporary DENV-1 strain from another region of Brazil and observed a significantly different pattern compared to both L1 and L6. Our data suggests antigenic differences between DENV-1 L1 and L6 that may contribute to the persistence of DENV-1 L6 lineage in SJRP.

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EVOLUTIONARY DYNAMICS OF DENGUE SEROTYPES REVEAL IMMUNITY-DRIVEN EPIDEMIC AMPLIFICATION IN BRAZIL

Tijs W. Alleman, Laura W. Alexander, Sanjana Bajaj, Ana I. Bento

Cornell University, Ithaca, NY, United States

Dengue fever, a mosquito-borne disease infecting 390 million people annually in (sub) tropical regions, is caused by four flavivirus serotypes (DENV-1-4). The dynamics of these four serotypes are shaped by antigenic cross-protection, competitive exclusion, and genotypic diversity, generating cycles of strain replacement that can trigger unexpectedly large epidemics. These cycles, in turn, reshape the population immunity landscape, feeding back to alter strain competitiveness. Understanding how serotype competition, population immunity, and transmission dynamics interact is crucial for anticipating epidemics and designing vaccination strategies. Here, we reconstruct serotype-specific dengue incidence in Brazil (1999-2025) to quantify the influence of population immunity on epidemic magnitude. We develop a spatiotemporal transmission model that tracks heterologous infections with transient cross-protection and permits homologous reinfection at reduced propensities, particularly following the introduction of novel genotypes. Within this mechanistic framework, negative frequency-dependent selection between serotypes emerges endogenously, enabling us to interpret serotype competition through the lens of evolutionary competition. The inferred latent serotype dynamics reveal a strong association between the introduction of novel genetic diversity and increases in the susceptible population, driving serotype replacement and large epidemics. These findings suggest that, beyond commonly emphasized climatic drivers, intrinsic immunological and evolutionary processes play a central role in shaping epidemic magnitude. We further identify homologous reinfection as a non-negligible contributor to dengue burden, including in regions not typically considered endemic. By integrating clustering, hierarchical Bayesian inference, mechanistic transmission modeling, and evolutionary dynamics, our framework provides a unified approach to extracting epidemiological signals from incomplete surveillance data and offers new insights into how the dengue immunity landscape shapes epidemic size.

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SEASONAL CLIMATE PERTURBATIONS INFORM DENGUE EPIDEMIC LENGTH TIMING AND MAGNITUDE GLOBALLY

Har-Kishen Joshi

London School of Hygiene and Tropical Medicine, London, United Kingdom

Dengue is a mosquito-borne disease of increasing public health significance. Incidence varies seasonally and country-specific dengue seasons vary globally due to differences in timing of climatic seasons. Importantly, intervention timing within the dengue season impacts efficacy, with the WHO recommending earlier intervention relative to the season peak to achieve greater case reduction. This work identifies windows within the dengue season whose climate is indicative of dengue season characteristics. To adjust for differences in dengue season timing globally, we developed a relative definition of seasonality that characterizes

climate by timing relative to the dengue seasonal cycle rather than to the calendar year. Dengue seasons were defined as lasting twelve months from the month with lowest cases on average. We modelled how changes to seasonal climate drive changes to dengue season characteristics, specifically peak timing, size and length. Peak timing was modelled as shift from average. Z-scores were used to express peak size and length, and seasonal climate as deviation from average. We modelled peak characteristics using climate at each phase of the dengue season to identify phases with potential to inform early warning systems. Rolling cross-validation was used to assess potential of changes in seasonal climate to forecast changes in dengue season features. Globally, preliminary analyses indicate that climate during season phase two, corresponding to three and four months since the month with lowest cases on average, are most influential in determining season peak size and timing. Higher-than-average rainfall is associated with later and smaller peaks, and increased temperature suitability is associated with larger peaks. This study provides the first global assessment of how changes to seasonal climate influence dengue season characteristics. We identify climatic windows within the dengue season with early-warning potential for changes to peak timing, length and size. These windows bear utility for decision making on intervention timing and provide evidence for using seasonal features in medium term forecasting.

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FLAVIVIRUS SEROPOSITIVITY AND SEROCONVERSION AMONG AGRICULTURAL WORKERS IN RURAL SOUTHWEST GUATEMALA

Blair W. Weikel[1], Julio David del Cid-Villatoro[2], Neudy Carolina Rojop[3], Daniela Castaño Vasquez[4], Cassandra Waltman[1], Claire Wickson Bradley[5], Chandler Bradley[1], Mattie Olivia Cassaday[6], Anika Cristina Hoffens Nunez[7], Lester Ivan Gutierrez Perez[8], Katherine Carillo[7], Molly Lamb[9], Daniel Olson[10]

[1]University of Colorado Anschutz Medical Campus, Aurora, CO, United States, [2]Fundación para la Salud Integral de los Guatemaltecos, Coatepeque, QZ, Guatemala, [3]Quetzaltenango, QZ, Guatemala, [4]Fundación Vive Con Bienestar, Manizales, Colombia, [5]Burlington, VT, United States, [6]Aurora, CO, United States, [7]Universidad del Valle de Guatemala, Guatemala City, Guatemala, [8]University of North Carolina, Chapel Hill, NC, United States, [9]Colorado School of Public Health, Aurora, CO, United States, [10]Univ of Colorado, Aurora, CO, United States

Dengue virus (DENV) is the highest burden arbovirus globally. Zika (ZIKV), a co-occurring flavivirus, increases infections and complicates serology. Because most DENV infections are asymptomatic, estimating population immunity remains challenging. Determining individual and communitylevel serostatus is essential for guiding DENV vaccine deployment, as current vaccines are recommended only for those with prior exposure. We analyzed serum blood samples from a longitudinal study of agricultural workers in southwest Guatemala (2020-2024), using an IgG-based multiplex bead assay conjugated to DENV1-4/ZIKV EDIII. An algorithm classified samples as having predicted naïve, monotypic, or multitypic immunity. We examined seropositivity and seroconversion for DENV or ZIKV and either flavivirus overall, exploring associations with demographic, occupational, and socioeconomic factors and conducting community-level geospatial analyses. Among 1,092 participants (80% male; median age 29) 96% demonstrated monotypic or multitypic flavivirus immunity. Degree of seropositivity varied by sex; overall seropositivity had no significant risk factors. Of the 286 workers who were flavivirus naïve or had monotypic immunity at baseline, 62% experienced a seroincident infection, though no significant risk factors were identified. Of those eligible for seroconversion at baseline, 58% and 18% had evidence of new DENV and ZIKV infections, respectively. Increased risk of DENV was associated with being a field foreman versus a field worker. Increased risk of ZIKV was associated with male sex and decreased risk associated living with some rural communities rather than the urban center. Geospatial analyses are in progress to identify community-level variations and climate impacts. Our findings reveal an exceptionally high burden of flavivirus infections among agricultural workers in this region. Given this population of young, healthy, primary income earners, they may benefit from targeted vaccination strategies. Improved understanding of local transmission dynamics can support more equitable and effective vaccine implementation.

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SEROPREVALENCE OF CHIKUNGUNYA VIRUS INFECTION IN BRAZIL BETWEEN 2023 AND 2025

Erika R. Manuli[1], Xinyi Hua[2], Gabriel C. Scachetti[3], Julia Forato[3], Ingra M. Claro[2], Geovana M. Pereira[1], Carla Dinardo[4], Fabiana Chagas[5], Luiz Amorim Filho[6], Luciane K. Schier[7], Allyson G. da Costa[8], Maria I. Valenca[9], Luciana M. Carlos[10], Marcelo Bragatte[11], Ligia M. Capuani[1], José L. Proenca-Modena[3], William M. de Souza[2], Ester C. Sabino[1]

[1]University of Sao Paulo, Sao Paulo, Brazil, [2]University of Kentucky, Lexington, KY, United States, [3]University of Campinas, Campinas, Brazil, [4]Fundacao Pro-Sangue/Hemocentro de Sao Paulo, Sao Paulo, Brazil, [5]Fundacao Centro de Hematologia e Hemoterapia do Estado de Minas Gerais – Hemominas, Belo Horizonte, Brazil, [6]Instituto Estadual de Hematologia Arthur de Siqueira Cavalcanti – Hemorio, Rio de Janeiro, Brazil, [7]Centro de Hematologia e Hemoterapia do Paraná - Hemepar, Curitiba, Brazil, [8]Hemoam - Fundação Hospitalar de Hematologia e Hemoterapia do Amazonas, Manaus, Brazil, [9]Hemope - Fundacao de Hematologia e Hemoterapia de Pernambuco, Recife, Brazil, [10]Centro de Hematologia e Hemoterapia do Ceara - Hemoce, Fortaleza, Brazil, [11]Instituto Todos pela Saude- ITpS, Sao Paulo, Brazil

Chikungunya virus (CHIKV) is a mosquito-borne alphavirus that causes approximately 17.7 million annual cases worldwide, and nearly 2.8 billion people live in areas at risk for infection. In this study, we combined serological, epidemiological, and ecological data to estimate population susceptibility to CHIKV infection and develop an evidence-based framework to prioritize CHIKV vaccines. Between January 1, 2014, and September 13, 2025, 597,572 laboratory-confirmed chikungunya cases were reported across 4,514 (81.04%) of 5,570 municipalities in all 27 federal units. Our seasonality analysis demonstrated that chikungunya transmission peaked between March and May, a pattern strongly associated with climate-driven Aedes aegypti. Next, we conducted cross-sectional seroprevalence surveys of anti-CHIKV IgG among 14,477 blood donors across seven Brazilian state capitals from November 2023 to June 2025. We found a crude overall seroprevalence of 14.2% (95% CI: 13.6–14.7, n=2,051 CHIKV-IgG seropositive) across all seven municipalities. Our findings align with national surveillance data: cities affected by major CHIKV outbreaks (Recife, Fortaleza, and Rio de Janeiro) exhibited high seroprevalence (14.3–42.9%), whereas cities without major epidemics (Manaus, São Paulo, Belo Horizonte, and Curitiba) showed lower rates (0.7–4.3%). Then, we used a Bayesian approach to quantify the CHIKV transmission potential index for all 5,570 Brazilian municipalities. Among the seven state capitals, we found a high CHIKV transmission potential index in Manaus, Recife, Rio de Janeiro, Fortaleza and Belo Horizonte, and lower levels in São Paulo and Curitiba, which mirrors the CHIKV epidemiological data. Notably, we found a strong positive correlation between the maximum monthly Index P values and observed seroprevalence (Pearson r = 0.8940). Next, we apply mathematical modeling to estimate the municipal-level susceptible population at higher risk of transmission to inform strategic CHIKV vaccine deployment. Collectively, these findings provide a comprehensive assessment of seroprevalence and transmission dynamics of CHIKV in Brazil.

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FACTORS ASSOCIATED WITH OUTBREAK DENGUE MORTALITY IN PRIMARY INFECTION DURING THE CYCLONE YAKU, IN LAMBAYEQUE, PERU 2023

Virgilio E. Failoc-Rojas[1], Mario Valladares-Garrido[2]

[1]Universidad San Ignacio de Loyola, Lima, Peru, [2]Hospital Regional Lambayeque, Lambayeque, Peru

Cyclone Yaku was an unusual tropical low-pressure system that formed in the Pacific Ocean. The dengue outbreak associated with Cyclone Yaku in

2023 led to an unusual increase in severe cases in northern Peru. However, the factors associated with mortality in primary infection in this context have not been adequately characterized. To identify the factors associated with mortality from primary dengue infection in patients hospitalized in Lambayeque-Peru, during the 2023 Cyclone Yaku outbreak. Retrospective case-control study of hospitalized patients with dengue. A total of 43 cases (deceased) and 86 controls (survivors) were included, matched in a 1:2 ratio. All diagnoses were confirmed by NS1 testing or IgM serology. Clinical, epidemiological, and laboratory variables were collected from medical records. Odds ratios (OR) with 95% confidence intervals were calculated using logistic regression. The following factors were identified as associated with mortality: age ≥60 years (OR: 4.82), duration of illness >6 days (OR: 5.54), markers of severe dengue (OR: 14.44), dyspnea (OR: 28.47), shock index ≥1 (OR: 9.47), and leukocytosis >15,000 (OR: 13.90). In conclusion, the main factors associated with mortality were clinical severity variables and delays in care, which highlights the importance of early recognition and timely management in outbreaks associated with extreme weather events.

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A COMPARISON OF SYNTHETIC NETWORK GENERATORS FOR RABIES MODELLING IN FREE ROAMING DOMESTIC DOGS

Inez Derkx[1], Nakul Chitnis[2]

[1]Swiss TPH, Allschwil, Switzerland, [2]Swiss Tropical & Public Health Institute, Allschwil, BL, Switzerland

Heterogeneous contact structures play a vital role in the transmission of canine rabies, yet empirical population networks are rarely fully observed. Synthetic network generators can therefore be used to reconstruct and scale observed networks, but the degree to which different generative algorithms can reproduce empirical contact heterogeneity and how this affects epidemic dynamics remains largely underexplored. Using an empirical spatial contact network of free-roaming domestic dogs (FRDD) in N'Djamena, Chad, we compare four different network generation approaches: a spatially explicit network construction algorithm, a stochastic block model, a degree-corrected stochastic block model, and an Erdős-Rényi random graph model as a null control. We evaluate how each generator reproduces key topological features, including degree distribution and clustering coefficients, of the empirical network, and how the resulting structural differences translate into variation in simulated outbreak dynamics. Each generator is further applied to multiple empirical FRDD networks of varying sizes to assess their generalizability. Our findings indicate that the degree-corrected stochastic block model best matches the empirical network both in overall network structure as well as in resulting rabies outbreak size and duration, highlighting the importance of incorporating both local and global network heterogeneity when constructing synthetic networks for rabies modelling and designing targeted intervention strategies. These results suggest that empirically informed network generators are promising tools for reconstructing and scaling empirical contact networks to simulate rabies outbreak dynamics in FRDD populations where contact data are incomplete or partially sampled.

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ACCEPTABILITY, FEASIBILITY, AND PERCEPTIONS OF SALIVA-BASED SPECIMEN COLLECTION ACROSS AGE GROUPS IN A LONGITUDINAL DENGUE HOUSEHOLD COHORT IN THAILAND

Ruchira R. Khosavanna[1], Rungkarn Kaenman[2], Adam Waickman[3], Kathryn B. Anderson[3], Darunee Buddhari[2]

[1]Yale University, New Haven, CT, United States, [2]Walter Reed Army Institute of Research - Armed Forces Research Institute of Medical Sciences, Kamphaeng Phet, Thailand, [3]SUNY Upstate Medical University, Syracuse, NY, United States

Sustainable longitudinal surveillance in field settings requires sampling approaches that are both feasible and acceptable to participants. While saliva collection offers a non-invasive alternative to phlebotomy, its successful integration into large-scale studies depends on community perceptions and usability across diverse age groups. We hypothesized that saliva collection is highly acceptable and easy to perform, with preferences varying by age and perspective. We conducted a mixed-methods study to assess perceptions and experiences of saliva collection within an ongoing longitudinal dengue household cohort in Kamphaeng Phet, Thailand. Study staff administered structured Thai questionnaires, utilizing Likert-scale, closed-ended, and optional open-ended responses. Mother-infant dyads underwent monthly saliva collection, while adults and older children participated in quarterly serologic surveillance. Staff collected infant samples; others performed self-collection. Data collection is ongoing; we anticipate analysis for 300 individuals by May 2026. To date, 65 respondents have been interviewed, representing 109 individuals: 21 mother-infant pairs, 40 adults, and 27 children. Preliminary findings show high acceptability: all respondents reported saliva collection was easy to perform with no discomfort. 97% reported no issues with collection; one family reported an unpleasant taste from the swab. 73.8% of adults had no preference between saliva and blood collection (6.6% preferred saliva; 19.7% preferred blood). Clearer preferences emerged in pediatric groups, with more chose saliva for infants (52.4%) and children (37.0%) than blood (4.8% and 18.5%, respectively). Preferences reflected convenience and minimal discomfort for saliva versus perceived greater diagnostic capability for blood. Saliva sampling is a feasible, well-tolerated approach for dengue surveillance in Thailand, particularly for pediatric populations. These findings will guide the implementation of non-invasive strategies to enhance participant retention and community engagement in long-term infectious disease research.

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DETECTION OF DIFFERENT SEROYPES OF NON-POLIO ENTEROVIRUSES IN SEWAGE/WASTEWATER IN GHANA

Abdulai Taufik

Ghana Armed Forces Health Service, Accra, Ghana

This research investigated the prevalence of non-polio enteroviruses (NPEVs) in sewage samples across seven regions in Ghana. Following World Health Organization (WHO) guidelines, the study aimed to contribute to surveillance efforts for these viruses, which play a crucial role in public health monitoring. Environmental samples were collected from various sites in seven regions, with a focus on sewage waters. To detect the present enteroviruses, culture studies were employed and the detected viruses were serotyped using sero-neutralization techniques to identify specific enteroviral strains. The study found an overall prevalence of 36.5% (58/159) of NPEVs in the collected sewage samples. Out of 58 positive culture samples, 45% were typable. The Greater Accra Region exhibited the highest number of typable samples, while the Northern Region reported the least. Additionally, eleven NPEV serotypes were identified, with Coxsackie B virus dominating across various districts and regions. The study also reported the presence of Coxsackievirus A7, potentially novel to West Africa. Echovirus 11, Echovirus 19, and Echovirus 12 were also prevalent, highlighting a diversity of serotypes. In conclusion, the study underscored the need for surveillance of NPEVs in Ghana, as over 30% of sewage samples contained these viruses. Coxsackievirus B predominated, emphasizing the importance of monitoring this specific serotype. The detection of a new serotype highlighted the necessity of ongoing surveillance to prevent potential outbreaks. Based on the findings, the implementation of robust surveillance measures for NPEVs and research are crucial to understanding the evolving landscape of enteroviruses in the Country and preventing potential public health threats.

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PREDICTIVE MODELING OF CLIMATE AND URBANIZATION DRIVERS OF *AEDES* MOSQUITO INVASION AND ARBOVIRAL EMERGENCE RISK IN NIGERIA

Justin O. Nwofe[1], Adamu I. Akyala[2]

[1]APIN Public Health Initiatives, Abuja, Nigeria, [2]Global Health and Infectious Diseases Control Institute Nasarawa State University, Keffi, Nigeria

Rapid urbanization and climate variability are reshaping the ecological distribution of *Aedes* mosquitoes, increasing the risk of arboviral disease emergence across Nigeria and Sub-Saharan Africa. This study developed predictive ecological models integrating climatic and urbanization indicators to identify areas at elevated risk for *Aedes* invasion and arboviral emergence in Nigeria. Species distribution models were developed using a Random Forest machine-learning framework integrating climatic predictors with urbanization indicators including population density and urban expansion proxies. Occurrence records for *Aedes aegypti* and *Aedes albopictus* were combined with environmental predictor layers to estimate habitat suitability across Nigeria. Model performance was evaluated using receiver operating characteristic analysis. Species-specific suitability maps were generated and overlaid to identify zones of vector co-existence associated with elevated arbovirus transmission risk. The models demonstrated excellent predictive performance (AUC = 0.993). Urbanization emerged as the strongest determinant of *Aedes* habitat suitability (MeanDecreaseAccuracy = 35.7), followed by temperature seasonality (19.1) and population density (17.4). Predicted suitability for *Aedes aegypti* was widespread across southern and central Nigeria, reflecting the species' strong association with densely populated urban environments. In contrast, *Aedes albopictus* suitability was concentrated primarily in humid southern regions and vegetated peri-urban zones. Spatial overlay of both suitability surfaces revealed distinct co-existence hotspots in southwestern Nigeria, the coastal belt, and parts of the Middle Belt transition zone, indicating areas of heightened potential for arboviral transmission. Urbanization and climate variability jointly drive the ecological expansion of *Aedes* mosquitoes in Nigeria. Predictive risk mapping provides a scalable framework for identifying emerging arboviral hotspots and strengthening early warning systems in rapidly urbanizing tropical settings.

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ASSESSING ZOONOTIC SPILLOVER RISK OF HENIPAVIRUSES AMONG BORDER SECURITY PERSONNEL IN SABAH, MALAYSIA

Sylvia Daim[1], Spencer Sterling[2], Lianying Yan[3], Saifulsyahira Jaaman[4], Ping-Chin Lee[1], Song-Quan Ong[1], Matthew Grigg[5], Nathaniel Christy[6], Christopher C. Broder[7], Dawn Weir[6], Eric Laing[8], Andrew G. Letizia[9]

[1]Universiti Malaysia Sabah, Kota Kinabalu, Malaysia, [2]HJF, Bethesda, MD, United States, [3]Clarksburg, MD, United States, [4]Malaysian Armed Forces (MAF), Kuala Lumpur, Malaysia, [5]Menzies School of Health Research, Darwin, Australia, [6]U.S. Naval Medical Research Unit INDO PACIFIC, Singapore, Singapore, [7]Uniformed Services Univ, Bethesda, MD, United States, [8]Uniformed Services University, Bethesda, MD, United States, [9]U.S. Naval Medical Research Unit, INDO PACIFIC, Singapore, Singapore

Security forces stationed along the international borders at the Malaysian North Borneo State of Sabah may be at increased risk of exposure to henipaviruses due to their operational proximity to known reservoir animal species. To assess the seroprevalence of henipavirus antibodies among this high-risk group, serum samples collected from cohorts of Malaysian soldiers before and after deployment were tested for IgG against soluble henipavirus receptor-binding (G) glycoproteins using a sensitive multiplex microsphere immunoassay (MMIA). The resulting antibody-antigen complexes were analyzed by cluster-based approaches to assess the binding profiles. Laboratory analysis is underway to test approximately 1200 paired serum samples collected between May 2023 and September 2024. In a preliminary analysis of serum from 361 soldiers, although minimal seroconversion occurred during deployment, post-deployment serum of two individuals was found to have IgG reactive to the shrew-associated Langya and Gamak viruses. This study will provide insights into the occupational risk of henipavirus exposure among border security forces, informing health protection measures and improving medical readiness. Results may also inform future biosurveillance measures and diagnostic evaluations to enhance regional biosecurity.

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SEROPREVALENCE AND SEROINCIDENCE OF *AEDES*-BORNE ARBOVIRAL INFECTIONS DURING PREGNANCY: RESULTS FROM THE PRISMA STUDY

Abigale Proctor[1], Emma Cook[1], Stacie Loisate[1], Jaime Marquis[1], Jamie Minchin[1], Agnes Oluoch[2], Arun Singh Jaduan[3], Asha Mary Abraham[4], Florence Achieng' Aweyo[5], John Fletcher[6], Aneeta Hotwani[7], Shayan Khakwani[7], Felistas Mbewe[8], Humphrey Mwape[8], Caleb Sagam[2], Neeraj Sharma[3], Meghna Singh[3], Nida Yazdani[7], PRISMA Consortium[9], Zahra Hoodbhoy[7], Muhammad Imran Nisar[10], Anne George Cherian[6], Santosh J. Benjamin[6], Victor Akelo[5], Charlotte Tawiah Agyemang[11], Kwaku Poku Asante[11], Sam Newton[11], Sarmila Mazumder[3], Bridget Spelke[12], Margaret P. Kasaro[8], Wilbroad Mutale[8], Emily Smith[1], Christopher N. Mores[1]

[1]George Washington University, Washington, DC, United States, [2]Kenya Medical Research Institute, Kisumu, Kenya, [3]Society of Applied Sciences, New Delhi, India, [4]Dept. of Clinical Virology, Christian Medical College, Vellore, Vellore, India, [5]Kisumu, Kenya, [6]Christian Medical College Vellore, Vellore, India, [7]Aga Khan University, Karachi, Pakistan, [8]UNC Global Programs Zambia, Lusaka, Zambia, [9]Prisma Consortium, [10]Aga Khan University, Karachi, SD, Pakistan, [11]Kintampo Health Research Center, Kintampo, Ghana, [12]UNC Chapel Hill, Chapel Hill, NC, United States

Aedes-borne viruses are a growing concern globally as outbreak frequency increases. Arbovirus cases are often missed or incorrectly identified due to frequent asymptomatic infection and overlapping, nonspecific clinical manifestation. Pregnant women are especially vulnerable, as these infections increase the risk of adverse outcomes. However, exposure among pregnant women is often ill-defined. As part of the Pregnancy Risk Infant Surveillance and Measurement Alliance (PRISMA) study, we estimated seroincidence and seroprevalence of three arboviruses across study sites in Ghana, Kenya, Zambia, Pakistan, northern India, and southern India. Using the Chembio ZCD rapid diagnostic test, pregnant women enrolled between March 2024 and May 2025 were tested for IgM and IgG antibodies against Zika (ZIKV), chikungunya (CHIKV), and dengue (DENV) at <20 weeks gestation and 32 weeks gestation. ZIKV had the lowest seroprevalence at all sites, with the highest IgG positivity reported at 33% in S India and lowest at 0% in Zambia. DENV IgG positivity was substantially higher in Asia, at 75-81% positivity, compared to 27.5% in Ghana, 3.9% in Kenya, and 0.8% in Zambia. CHIKV seroprevalence was more heterogenous, but generally higher in Asia, with 38-52% seropositivity, than in Africa, with 28% in Kenya, 21% in Ghana, and 1.3% in Zambia. Baseline IgM positivity was highest in S India for CHIKV (13%), in Ghana for DENV (14%), and in Kenya for ZIKV (2.6%). Every site saw seroincidence, defined by new IgM positivity at 32 weeks gestation, of all three arboviruses. Ghana had the highest ZIKV seroincidence at 0.91%, and Pakistan had the highest DENV and CHIKV seroincidence at 2.1% and 5.5%, respectively. Confirmatory testing is needed to distinguish true infection from cross-reactivity, especially between DENV and ZIKV. This serosurvey emphasizes high endemic arbovirus exposure and evidence of ongoing infection during pregnancy across all PRISMA sites. To mitigate disease exposure and risk of adverse outcomes in pregnancy, it is critical to strengthen broad disease surveillance and control measures, as well as specific antenatal surveillance and risk monitoring.

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HISTORICAL AND GEOSPATIAL PATTERNS OF FILOVIRUS SPILLOVER AND OUTBREAK AMPLIFICATION, 1967-2025

Sarah Gibes[1], Madison Kurth[1], Kelsee Baranowski[2], John Uelmen[3]
[1]University of Wisconsin-Madison, Madison, WI, United States, [2]Penn State University, University Park, PA, United States, [3]Madison, WI, United States

Filoviruses, including Marburg and Ebola viruses, are among the deadliest zoonotic pathogens. Since the first recognized Marburg outbreak in 1967, recurring spillover events and secondary amplification have caused major outbreaks with high case fatality rates. However, the spatial and ecological conditions that shape spillover and outbreak severity remain unclear. We are conducting a PRISMA-guided systematic review of published filovirus spillover and outbreak reports from 1967 to 2025. Primary studies describing filovirus detection, suspected spillover, or outbreaks in humans or animal hosts are being screened by two reviewers. For each event, we are extracting virus, year, location, host, suspected reservoir or exposure setting, transmission context, and outbreak outcomes, when available. Events are classified by likely spillover interface and amplification pathway and georeferenced for spatial analysis. Our growing database and map include hundreds of confirmed or suspected Ebola virus and Marburg virus records in humans and wildlife across sub-Saharan Africa, alongside the home range of the Egyptian fruit bat (Rousettus aegyptiacus), a probable wildlife reservoir. This geospatial synthesis is designed to identify recurrent exposure settings, geographic clustering, and areas of overlap between reported epizootic and zoonotic filovirus events and the ecology of wildlife reservoirs. By integrating historical outbreak data with wildlife range information, this study will help clarify where spillover risk may concentrate and support more targeted surveillance, preparedness, and rapid containment in recurrent filovirus landscapes.

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IGM RESPONSES TO MOSQUITO SALIVARY PROTEINS DEMONSTRATE ASSOCIATIONS WITH RECENT WEST NILE VIRUS EXPOSURE IN A NON-HUMAN PRIMATE MODEL

Cameron Poss, Alyssa R. Schwinn, Kaitlyn Lewis, Claudia P. Herrera, Eric O. Dumonteil, Berlin Londoño-Renteria
Tulane University, New Orleans, LA, United States

Arthropod-borne viruses are a persistent threat to global health, with mosquitoes serving as vectors that sustain their transmission. West Nile Virus (WNV) is endemic in North America, where mosquito species such as *Culex quinquefasciatus* and *Aedes albopictus*, act as established vectors for many viral pathogens. Measurement of IgG antibodies against vector saliva has been widely used to characterize host-vector-pathogen interactions in human populations. However, despite the potential to indicate acute exposure, the role of IgM antibodies in estimating vector exposure has been largely overlooked. Understanding immune responses to pathogens and vector antigens in validated animal models can inform surveillance strategies to improve prediction of epizootic transmission, advance understanding of infection dynamics, and contextualize vector ecological niches. Consequently, we aimed to measure IgM responses against *Cx. quinquefasciatus* and *Ae. albopictus* salivary proteins, as well as WNV antigen, using our validated indirect ELISA test on 135 serum samples collected from 2019-2021 from 40 non-human primates housed at the Tulane National Biomedical Research Center (TNBRC). Although no overall difference in IgM responses between *Ae. albopictus* and *Cx. quinquefasciatus* was observed ($p = 0.1278$), antibodies against *Ae. albopictus* salivary proteins were more strongly associated with recent exposure to WNV than those to *Cx. quinquefasciatus* ($\rho = 0.5725$, $p < 0.0001$; $\rho = 0.25494$, $p = 0.003$), consistent with IgG trends in the same population and local avian surveillance data. Temporal analysis of IgM levels revealed greater year-to-year variability in IgM titers to *Cx. quinquefasciatus*, whereas responses to *Ae. albopictus*and WNV remained relatively stable. Although not significant, IgM responses to WNV showed a positive trend with age, mirroring human epidemiological patterns ($\rho = 0.13$, $p = 0.15$). These findings suggest that IgM may serve as a reliable indicator of vector and WNV exposure in Louisiana. Future work will examine seasonal IgM dynamics at the vector-pathogen interface using an expanded cohort over a longer sampling period.

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HETEROTYPIC ANTIBODY-DEPENDENT COMPLEMENT ACTIVATION AGAINST DENGUE VIRUS ENVELOPE AND NS1 ANTIGENS DIFFERENTIATES CLINICAL OUTCOMES IN SECONDARY DENV3 INFECTIONS

Amro Nasser, Priscila Da Silva Castanha, Ernesto T.A. Marques
University of Pittsburgh, Pittsburgh, PA, United States

Dengue viruses (DENV1-4) are four serologically distinct arboviruses responsible for dengue fever (DF) and the more severe dengue hemorrhagic fever (DHF). Most infections are self-limited, but a subset progresses to DHF, characterized by plasma leakage and risk of cardiovascular shock. Disease severity is driven by dysregulated inflammatory responses, in which complement activation plays a major role through the release of complement anaphylatoxins that activate the endothelium to increase vascular permeability. However, the contribution of antibody-dependent complement activation (ADCA) to disease outcome remains poorly defined. Using sera from a well-characterized DENV3 patient cohort, samples from primary and secondary DENV3 infections, classified as DF or DHF, were analyzed across febrile, critical, and recovery phases. A bead-based assay quantified ADCA against DENV3 envelope (E) and non-structural protein 1 (NS1), as well as heterologous DENV1/2 antigens in secondary infections, while patient complement protein levels were measured by ELISA. We observed ADCA patterns that were specific to the antigen and clinical outcome. Patients with DF show an early shift towards more ADCA targeting the DENV3 E protein, which suggests a protective response that is focused on the homotypic virus. Conversely, patients with DHF don't have an early, focused response to the DENV3 E protein, and show more ADCA directed to the DENV1/2 E protein. Instead, DHF patients are marked by ADCA profiles that reflect a sudden increase in anti-DENV3 NS1 ADCA during the critical phase, compared to DF patients. Pro-inflammatory complement anaphylatoxin levels were found to correlate with this increase in ADCA. In addition, using Principal Component Analysis to combine ADCA data and patient complement parameters allows us to clearly separate secondary DENV3 patients into different groups based on their clinical outcome. Combined, this data demonstrates that ADCA profiles are associated with clinical outcomes and mechanistically linked to complement activity, suggesting that the complement cascade may be a target for therapeutic development.

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ELISA-BASED DENGUE SEROPREVALENCE UNDERESTIMATES NEUTRALIZING ANTIBODY RESPONSES IN MOTHERS FROM NIGER

Bachir Assao Neino[1], Luke Trimmer-Smith[2], Ruiyu Pu[2], adriana gallagher[3], Michelle Papa[4], Ousmane Guindo[5], Boureima Hassane[6], Matt Hitchings[7], Derek Cummings[3]
[1]UF Emerging Pathogens Institute and Biology Department, Gainesville, FL, United States, [2]UF Emerging Pathogens Institute, Gainesville, FL, United States, [3]Johns Hopkins University, Baltimore, MD, United States, [4]Baltimore, MD, United States, [5]Epicentre Epidemiology, Niamey, Niger, [6]University of Maradi, Maradi, Niger, [7]Department of Biostatistics, University of Florida, Gainesville, FL, United States

Serologic surveys are essential for tracking dengue virus (DENV) transmission and immunity, especially where surveillance is sparse. While ELISA is a standard tool for large-scale studies, its capacity to measure past infection and functional immunity is uncertain—particularly for results near diagnostic cut-offs. This gap is critical in Niger, where dengue data are limited. We screened anti-DENV IgG in 200 mothers (aged 14-45) using a commercial indirect ELISA (EUROIMMUN) coated with recombinant NS1 from DENV serotypes 1-4. Seroprevalence was calculated using manufacturer cut-offs for non-endemic (10 RU/ml) and endemic (20 RU/ml) settings. Plaque reduction neutralization tests (PRNT) were performed

on 33 samples to detect specific neutralizing antibodies. We assessed agreement between methods, ELISA reproducibility through duplicates, and a two-component mixture model to capture measurement uncertainty. The choice of threshold markedly affected results: seroprevalence was 52.0% at 10 RU/ml but 28.5% at 20 RU/ml. Four PRNT-positive mothers were classified as negative by the endemic cut-off, clustering near the diagnostic threshold. Duplicate tests showed high reproducibility. The mixture model indicated substantial uncertainty around cut-offs, suggesting its utility for prioritizing confirmatory testing rather than defining serostatus. We used this model to estimate the hazard of infection of dengue in this population. ELISA-based surveys may underestimate dengue immunity in individuals with low-to-moderate antibody levels. In Niger, reliance on fixed cut-offs may understate past exposure among women of childbearing age. Accounting for diagnostic uncertainty is essential when interpreting dengue serologic data in understudied settings.

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DEVELOPMENT OF A MULTIPLEX IMMUNOASSAY USING RECOMBINANT SUBUNIT ANTIGENS FOR THE DETECTION OF FILOVIRUS-SPECIFIC IMMUNE RESPONSES IN NON-HUMAN PRIMATES

Isabelle E. Eiser, Axel T. Lehrer, Teri-Ann Wong, Brien Keith Haun, Vivek R. Nerurkar

John A Burns School of Medicine, University of Hawaii Manoa, Honolulu, HI, United States

Ebola virus (EBOV), and other *Filoviruses,* are BSL-4 pathogens, causing significant threats to global public health as a biological weapon or through imported infections from international travel. Predicting and preventing EBOV outbreaks has been difficult as outbreak control is dependent on early identification of viral circulation in a region combined with rapid diagnosis of infected patients. Serological assays have revealed EBOV exposure in an array of mammals including orangutans, fruit bats, pigs, and even dogs with overlapping endemicity. The genetic variability of EBOV, combined with the lack of a known reservoir, highlights the need for a robust, specific, and rapid assay that focuses on detecting an antibody response in relevant species of animals and humans. This assay may serve as a stable proxy for the prevalence of EBOV infection in endemic areas. Multiplexed platforms offer multi-antigen detection in a single well, increasing the amount of data generated per test. Here, we describe the development and testing of a Luminex technology-based Microsphere Immunoassay (MIA) for detection of EBOV and other filovirus antigen-specific IgG and IgM responses. Soluble recombinant Filovirus surface glycoproteins (GP) and matrix proteins of EBOV (VP24 and VP40) were generated in the *Drosophila* S2 cell expression system, purified by immunoaffinity chromatography and covalently coupled to magnetic microspheres. We compared the performance of the newly developed MIA to a conventional ELISA for detecting EBOV and other filovirus-specific antibodies using serum samples from a vaccine efficacy study in rhesus macaques and found single-point MIA specificity and sensitivity were comparable to standard 8-fold dilution IgM/IgG ELISA titers while saving substantial amounts of sample volume and time. This assay has over time proven utility in testing vaccinated populations of non-human primates and humans with comparable results to the gold standard Filovirus Animal Non-clinical Group ELISA assay. Further, this platform has shown utility in human populations that have been previously infected with Marburg virus

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DIFFERENTIAL SUSCEPTIBILITY TO ANTIBODY-DEPENDENT ENHANCEMENT OF DENGUE VIRUS INFECTION IN SKIN-RESIDENT MYELOID CELLS BETWEEN EUROPEAN AND AFRICAN ANCESTRY DONORS

Jacqueline Holden, Michelle Martí, Aleena Alex, Isabelle Kahng, Ernesto T.A. Marques, Simon Barratt-Boyes, **Priscila MS Castanha**

University of Pittsburgh, Pittsburgh, PA, United States

Dengue virus (DENV) infection outcomes range from asymptomatic to severe disease, often driven by antibody-dependent enhancement (ADE) during secondary heterotypic infections. Our previous research using human skin explants suggests that European ancestry (EA) is associated with increased DENV infection and inflammatory responses compared to African ancestry (AA). However, the underlying host mechanisms for these differences under enhancing conditions remain unexplored. We investigated the expression of Fc-gamma receptors (FcγR) and compared ADE susceptibility in primary dermal cells from EA and AA donors. Dermal cells were isolated from skin donors and analyzed via flow cytometry for FcγR expression (CD32, CD64) and lineage markers (CD45 for leukocytes, CD163 for macrophages, CD1c for dendritic cells). We infected isolated cells with DENV-2 without serum or pre-incubated with varying dilutions of DENV-3 specific serum. Infection and cellular markers were analyzed 24 hours post-infection. EA donors exhibited significantly higher median fluorescent intensity for CD64 compared to AA donors, though no other baseline differences in FcγR expression or cell population frequencies (CD45+, CD163+, CD1c+) were observed. Under primary infection conditions (DENV without antibodies), EA donors showed a trend toward higher DENV+ cell frequencies, though this did not reach statistical significance. Peak ADE occurred at a 1:100 serum dilution, with the greatest enhancement observed in macrophages and dendritic cells. While ADE was present in both groups across all serum dilutions, EA donors demonstrated significantly higher ADE levels, particularly at 1:10 and 1:100 serum dilutions. These results indicate a greater susceptibility of EA-derived myeloid cells to DENV infection under enhancing conditions. Understanding these ancestry-linked mechanisms of secondary infection is critical for identifying high-risk populations, improving clinical risk stratification, informing vaccine deployment strategies and developing targeted therapeutics for the billions at risk of dengue disease.

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NEUTROPHIL PHENOTYPES AND FUNCTION IN ACUTE DENGUE AND ASSOCIATIONS WITH DISEASE SEVERITY

Camila D. Odio[1], Heather L. Teague[2], Chanthap Lon[3], Heng Seng[4], Ny Chanty[5], Sovann Ly[6], Rithea Leang[7], Rekol Huy[8], Kimsreng Lean[9], Piseth Ly[10], Sophana Chea[11], Sreytouch Chea[12], Mengheng Oum[12], Sreynik NHEK[10], Sokna Ly[12], Ratanak Sath[12], Pich Chea[12], Chiny Voeurn[12], Sreyvouch Pat[12], Rany Vorn[13], Somnang Man[14], Sreyngim Lay[11], Tineke Cantaert[15], Christina Yek[16], Jeffrey R. Strich[17]

[1]Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States, [2]Critical Care Medicine Department, National Institutes of Health Clinical Center; Critical Care Medicine and Pulmonary Branch, National Heart Lung and Blood Institute, Bethesda, MD, United States, [3]International Center of Excellence in Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Phnom Penh City, Cambodia, [4]Surveillance Program, Phnom Penh, Cambodia, [5]Preah Kossomak Hospital, Phnom Penh, Cambodia, [6]Communicable Disease Control Department, Ministry of Health, Phnom Penh, Cambodia, [7]Natl Ctr for Parasitology, Entomology, and Malaria Control, Phnom Penh, Cambodia, [8]National Ctr for Parasitology Entomology & Malaria Control, Phnom Penh, Cambodia, [9]National Pediatric Hospital, Phnom Penh, Cambodia, [10]ICER Cambodia, Phnom Penh, Cambodia, [11]International Center of Excellence in Research, Phnom Penh, Cambodia, [12]International Center of Excellence in Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Phnom Penh, Cambodia, [13]Immunology Unit, Institut Pasteur du

Cambodge, Pasteur Network, Phnom Penh, Cambodia, [14]International Center of Excellence in Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, phn, Cambodia, [15]Institut Pasteur du Cambodge, Phnom Penh, Cambodia, [16]NIH, Phnom Penh, Cambodia, [17]Critical Care Medicine Department, National Institutes of Health Clinical Center; Critical Care Medicine and Pulmonary Branch, National Heart Lung and Blood Institute; United States Public Health Service Commissioned Corps, Bethesda, MD, United States

Despite its growing global burden, there are no approved dengue therapies. While neutropenia is a feature of dengue, it does not predict severity. In contrast, neutrophil activation has been associated with severe disease, suggesting that defining neutrophil phenotypes and function may inform pathogenic mechanisms and therapeutic targets. Low density neutrophils (LDNs) are a subset of neutrophils that localize to the peripheral blood mononuclear layer (PBMC) following density gradient centrifugation and are pathogenic and hyperactive. We enrolled n=113 hospitalized patients with laboratory confirmed dengue within 72 hours of symptom onset in Cambodia (n=96 had dengue fever (DF) and n=17 with dengue hemorrhagic fever/shock syndrome (DHF/DSS). Age and sex matched healthy controls (n=14) were enrolled for comparison. Samples were collected at days 0, 1, 3 and analyzed by complete blood count (CBC) and flow cytometry with 10 extracellular and 2 intracellular markers from the whole blood and PBMC fraction (for LDNs). Minimum absolute neutrophil counts, from the CBC, did not differ by severity group (DF: median 1.1, IQR 0.8 - 1.5 x10^9/L vs. DHF/DSS 1.3, 1.2-1.9 x10^9/L). Consistent with the CBC data, initial analyses of flow cytometry data revealed similar percentages of whole blood neutrophils from healthy donor patients and dengue patients with DF and DHF/DSS. However, dengue patients had a significantly higher percentage of LDNs than healthy controls (dengue day 0 median LDN 6%, IQR 3-17% vs. healthy control 1%, 1-5%, $p<0.001$), suggesting hyperactive neutrophils may contribute to pathogenesis. Lastly, we found that the percentage of LDNs decreased between days 0 and 3 indicating that neutrophils associate with the acute pathology and return to homeostasis as patients improve. Ongoing work will evaluate associations between disease severity and additional neutrophil signatures including cytokine signaling and markers associated with neutrophil extracellular trap formation and degranulation. Comparisons across severity groups, time, and diseases will inform how these cells influence disease and guide intervention strategies.

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CYD-TDV VACCINATION RESHAPES DENGUE ANTIGENIC RECOGNITION IN BASELINE-SEROPOSITIVE CHILDREN IN PUERTO RICO

Courtney Abad Micheletti[1], Samantha Osman[2], Fabiola Angelise Rodriguez Alvarado[3], Rachel Marie Rodriguez[4], Vanessa Rivera-Amill[5], William Messer[6]

[1]Oregon Health and Sciences University, Portland, OR, United States, [2]Oregon Health & Science University, Portland, OR, United States, [3]Ponce, Puerto Rico, [4]Yauco, PR, United States, [5]Ponce Medical School Foundation, Inc., Ponce, PR, United States, [6]Oregon Health & Sciences University, Portland, OR, United States

Vaccination with CYD-TDV induces neutralizing antibodies (NAbs) to all four dengue virus (DENV) serotypes in most recipients after a 3-dose series. However, protection is serotype-dependent and varies as circulating viruses diverge from the tetravalent vaccine components. Because even limited amino-acid variation in the envelope (E) protein can significantly shift neutralization, it remains unclear how CYD-TDV shapes the specificity and breadth of functional antibody recognition across antigenically and genetically diverse DENV strains. We analyzed sera from seven dengue-seropositive pediatric participants recruited in San Juan, Puerto Rico (ages 9–16) who completed the vaccine series. Neutralization titers were quantified by FRNT50 at baseline and at 7, 14, and 28 days after each dose/boost. Neutralization breadth was profiled at baseline and at 28 days post–dose 3 against a panel of antigenically and genetically diverse DENV strains spanning multiple genotypes within each serotype; pre- versus post-vaccination serum–virus relationships were modeled using antigenic cartography. Antibodies to YFV 17D NS1 were assessed longitudinally across interim time points by ELISA, and anti-DENV NS1 responses were measured at baseline and post-vaccination. Longitudinal FRNT analysis found substantial heterogeneity in boosting across participants and serotypes. Titer increases were more pronounced for DENV1 and DENV2, whereas DENV3 and DENV4 responses were smaller and more variable; in some participants, DENV3 and/or DENV4 did not exceed a ≥4-fold boost threshold. Antigenic cartography further indicated vaccination-associated shifts in antigenic recognition, consistent with remodeling of the functional antibody repertoire. In baseline-seropositive children receiving CYD-TDV, vaccine-associated changes in neutralization are most interpretable in an antigenic framework that captures how antibodies engage viruses across strain space rather than genetic distance alone. These findings support functional, antigenically informed evaluation of CYD-TDV–elicited immunity against diverse circulating DENV strains.

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IMMUNE PROFILING OF CYD-TDV DENGUE VACCINE RECIPIENTS USING NEUTRALIZATION ASSAYS AND NEUTROPHIL TRANSCRIPTOMICS

Fabiola Angelise Rodriguez Alvarado[1], Rachel M. Rodriguez-Santiago[1], Raphael Sánchez[1], Andrea Arias-García[1], Paola N. Flores Pérez[1], Adrián Marquez[1], Andrea Paola Rivera-Torres[1], Mercedes Y. Lacourt[1], Marcos Javier Ramos-Benitez[1], William Messer[2], Vanessa Rivera-Amill[1]

[1]Ponce Health Sciences University, Ponce, PR, United States, [2]Oregon Health & Sciences University, Portland, OR, United States

Dengue virus (DENV) remains a major public health burden in endemic regions such as Puerto Rico. The tetravalent dengue vaccine CYD-TDV (Dengvaxia®) is recommended for DENV-seropositive individuals aged 9-16; however, the immunologic mechanisms underlying protective responses remain incompletely defined. Integrative analyses of humoral and innate immune responses may help identify correlates of protection. We aimed to characterize longitudinal immune responses following vaccination in DENV-seropositive individuals. We enrolled 11 participants. Peripheral blood samples were collected at baseline and days 7, 14, and 28 following the first vaccine dose. Preliminary analyses were conducted in a subset of two participants. Anti-DENV IgG and IgM were measured by ELISA, and neutralizing antibody responses were quantified using focus reduction neutralization tests ($FRNT_{50}$). Innate immune profiling was performed on isolated neutrophils using bulk RNA sequencing to assess early transcriptional responses. ELISA analyses demonstrated increasing anti-DENV IgG levels over time, consistent with boosting of pre-existing immunity. Neutralizing antibody responses ($FRNT_{50}$) increased longitudinally, with the highest responses observed by day 28. DENV-1 and DENV-4 serotype-specific differences were observed, with stronger neutralization against DENV-1. Baseline seropositivity was associated with detectable neutralizing activity prior to vaccination. Neutrophil transcriptomic profiling revealed a temporally structured response characterized by early downregulation (Day 7) followed by delayed activation (Day 14), consistent with a biphasic pattern. These data suggest robust humoral responses following CYD-TDV vaccination, with preliminary evidence of temporally structured innate immune changes and serotype-specific heterogeneity. Integrated immune profiling may provide insights into potential correlates of protection and help inform dengue vaccine strategies in endemic populations.

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HUMORAL IMMUNE SIGNATURES AS SURROGATES OF FUNCTIONAL NEUTRALIZATION FOR FLAVIVIRUS CLASSIFICATION IN CO-CIRCULATION SETTINGS

Cynthia G. Lorang[1], Jonathan P. Bashor[2], Madeline E. Kernahan[3], Iris Anne C. Reyes[4], Magdalena E. Pomichowski[4], Sara Y. Tartof[4], Gregory S. Davis[4], Joseph S. Lewnard[5], Christopher N. Mores[6]

[1]Milken Institute School of Public Health at The George Washington University, Wa, DC, United States, [2]George Washington University - Mores

Lab, Washington, DC, United States, [3]Milken Institute School of Public Health at The George Washington University, Washington, DC, United States, [4]Kaiser Permanente Southern California, Pasadena, CA, United States, [5]University of California, Berkeley, Berkeley, CA, United States, [6]George Washington University, Washington, DC, United States

Emerging *flaviviral* outbreaks threaten immunologically naïve populations as climate change and urbanization drive vector expansion into non-endemic regions, like S. California. Co-circulating dengue (DENV) 1-4 and West Nile (WNV) pose epidemiologic and diagnostic challenges, as short viremic periods and heterogeneous immunity contribute to antibody cross-reactivity, limiting specificity. Laboratory diagnosis relies on RT-PCR and serology, yet conventional assays lack specificity to distinguish closely related *flaviviruses.* Neutralization assays (PRNT, FRNT) provide gold-standard confirmation of exposure and immunity but require specialized infrastructure, limiting scalability for surveillance. We hypothesized that serological immune signatures could serve as surrogate predictors of functional neutralization, enabling scalable classification. Acute and convalescent serum samples were evaluated using FRNT against DENV1, DENV3, and WNV alongside ELISA for IgG, IgM, and NS1. Neutralization curves calculated $FRNT_{50}$ titers. Receiver operating characteristic analysis assessed whether serological markers predict neutralization ($FRNT_{50}$ ≥40) and correlate with clinical markers, with performance quantified by AUC and Youden's Index. IgG predicted neutralization (AUC=1.00, p=0.004), concordant with FRNT as functional immunity surrogate; DENV+/WNV– seroreactivity. IgM and NS1 showed good predictive ability (AUC=0.86, 0.83; p=0.037, 0.088), reflecting early-phase kinetics. $FRNT_{50}$ correlated strongly with IgG (AUC=0.88, p=0.031) and moderately with IgM and NS1, indicating complementary detection of distinct infection stages. $FRNT_{50}$ identified polyclonal neutralization across DENV serotypes, distinguishing co-infection or sequential exposure from primary infection. These findings support tiered diagnostics with IgM/NS1 for acute detection and IgG for neutralization. Adding multiplex serology will define humoral signatures for species and serotype differentiation in low co-circulating *flaviviruses* regions, improving scalability and outbreak detection in resource-limited settings.

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TRANSFORMING GROWTH FACTOR B1 (TGFB1) AS AN IMPORTANT IMMUNOMODULATOR IN THE PROTECTION AGAINST SEVERE THROMBOCYTOPENIA ASSOCIATED WITH DENGUE.

Maria Fernanda Yasnot-Acosta[1], Margarita Diaz[2], Maria Camila Velasco Pareja[2]

[1]GIMBIC, Universidad de Córdoba; Laboratorio de Salud Publica, Universidad de Córdoba, Monteria (Cordoba), Colombia, [2]GIMBIC, Universidad de Córdoba, Monteria (Cordoba), Colombia

Dengue is an arboviral disease with a high incidence in tropical regions, and its impact has intensified in Colombia, especially in the department of Córdoba. Thrombocytopenia is a frequent clinical manifestation and has been linked to alterations in the cytokine-mediated immune response. IL-6, IL-10, and TGF-B1 have been implicated in inflammatory and regulatory processes during the acute phase of dengue virus infection. Seventy samples were obtained from patients diagnosed with dengue and 50 healthy controls. Clinical, demographic, and hematological variables were collected. Serum concentrations of IL-6, IL-10, and TGF-B1 were quantified by flow cytometry. The most frequent symptoms were fever (100%), headache (77.1%), and myalgia (67.1%). Severe thrombocytopenia (<50 × 10^9/L) was highly prevalent (55.4%). Dengue with warning signs accounted for 51.4% of cases, followed by dengue without warning signs (45.7%) and severe dengue (2.9%). A significant negative correlation was observed between clinical severity and platelet count ($\rho = -0.618$; $p < 0.001$). IL-6 and IL-10 showed elevated concentrations in all clinical groups compared to healthy controls ($p < 0.001$), with no significant differences between the different degrees of thrombocytopenia. TGF-B1 concentrations were low in patients with severe thrombocytopenia ($p < 0.001$), lower than in the control group. Severe thrombocytopenia was frequently observed in the dengue patients studied, with a predominant pro-inflammatory immune response mediated by IL-6 and IL-10, regardless of the degree of thrombocytopenia. TGF-B1 appears to be an important molecule for preventing severe dengue.

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SEQUENTIAL, BUT NOT ISOLATED, YELLOW FEVER VIRUS-ASSOCIATED IMMUNE HISTORIES PRODUCE TITER-DEPENDENT ENHANCEMENT OF ZIKA AND DENGUE VIRUSES

Sebastian Gallon[1], Prince Baffour Tonto[1], Yifeng Ding[1], Wei-Kung Wang[2], Bobby Brooke Herrera[1]

[1]Rutgers Global Health Institute, Rutgers University, New Brunswick, NJ, United States, [2]Department of Tropical Medicine, Medical Microbiology and Pharmacology, John A. Burns School of Medicine, University of Hawaii at Manoa, Honolulu, HI, United States

Antibody-dependent enhancement (ADE) influences disease severity across orthoflavivirus infections, yet how layered exposure histories shape the balance between neutralization and enhancement remains incompletely defined. This question is particularly relevant in regions where yellow fever virus (YFV) infection or vaccination overlaps with endemic Zika virus (ZIKV) and dengue virus (DENV) circulation. Here, we combined serocomplex-resolved serology with functional assays to link defined exposure profiles to ADE phenotypes in a human cohort. Using anti-prM Western blotting together with microneutralization against YFV-17D, DENV-1-4, ZIKV, and West Nile virus (WNV), plasma was stratified into YFV-only, YFV + DENV, and YFV + DENV + ZIKV groups. Although E and NS1 binding was broadly cross-reactive across groups, neutralization breadth expanded with cumulative exposure. In Fcγ receptor-bearing U937 cells, enhancement activity was minimal in YFV-only plasma but increased following sequential exposure, with robust ADE across multiple heterologous viruses. To assess in vivo relevance, plasma was passively transferred into *IFNAR1*$^{-/-}$ mice at low (2 µl) or high (100 µl) doses prior to ZIKV or DENV-2 challenge. YFV-only plasma did not enhance infection in vivo. In contrast, YFV + DENV plasma significantly increased ZIKV viremia and worsened clinical parameters. YFV + DENV + ZIKV plasma exhibited a concentration-dependent phenotype, enhancing ZIKV at low dose but not high dose. Following DENV-2 challenge, sequential exposure groups demonstrated concentration-dependent modulation of early viral kinetics, with low-dose transfer trending toward increased viremia and high-dose transfer shifting toward attenuation. These findings indicate that YFV exposure alone does not predispose to ADE; rather, cumulative orthoflavivirus exposure generates antibody repertoires that create concentration-dependent risk windows in vivo.

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THERAPEUTIC EFFICACY OF ARTEMETHER LUMEFANTRINE IN THE TREATMENT OF UNCOMPLICATED *PLAMODIUM FALCIPARUM* MALARIA IN MSAMBWENI SUB-COUNTY, COASTAL KENYA

Kelvin K. Thiongó[1], Lewis Karani[1], Miriam Chelangat[1], Mary Ombati[1], Maureen Otinga[1], Francis T. Kimani[1], Sepha Mabeya[2], Damaris Matoke-Muhia[1], Daniel Muthui Kiboi[2]

[1]Kenya Medical Research Institute, Nairobi, Kenya, [2]Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya

Malaria remains a leading cause of morbidity in Kenya, with the Coastal region experiencing a high disease burden. Despite the World Health Organization's recommendation of Artemisinin-based Combination Therapies (ACTs), reports of partial resistance are emerging across Sub-Saharan Africa. This study evaluated the therapeutic efficacy of Artemether-lumefantrine (AL), Kenya's first-line treatment, in Msambweni, Kwale County. A one-arm prospective therapeutic efficacy study (TES) was conducted based on the standard WHO 42-day protocol. Children aged 6 months to 14 years with microscopically confirmed uncomplicated *P. falciparum* malaria were enrolled. Participants received a supervised three-day course of AL and were monitored on days 1, 2, 3, 7, 14, 21, 28, and 42. Primary endpoints were Adequate Clinical and Parasitological Response (ACPR) at days 28 and 42. Of 1,189 patients screened, 166 were enrolled (median

age: 68 months; median parasitemia: 25,000/µl). Two participants reported parasitemia on Day 3, which were confirmed recrudescent after genotyping, although they harbored wild type parasites after *Plasmodium Pfk13* sequencing. By Day 3, 92.8% of participants had cleared their parasites, with a median clearance time of 2 days. The uncorrected Intention-to-Treat (ITT) results were comparable, with an ACPR of 80.2% at Day 42. After PCR correction, the treatment efficacy of Artemether lumefantrine increased. The PCR corrected ACPR at day 42 in the ITT population was 94.8% (95% CI:91.4-98.4) while at day 28 PCR corrected ACPR was 92.6% (95% CI: 87.2-98.3). In conclusion, the efficacy of AL in Msambweni is on the brink of falling below the WHO-mandated 90% threshold for first-line antimalarials. These findings indicate a significant decline in AL therapeutic response in Coastal Kenya. Presence of participants with Day 3 parasitemia and recrudescence on other follow-up days is an indicator of drug failure. Continuous surveillance and molecular characterization of resistance markers are urgently required to inform national malaria treatment policy.

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COUNTRYWIDE PREVALENCE OF ANTIMALARIAL DRUG RESISTANCE MARKERS IN MAINLAND TANZANIA: A 2023 HEALTH FACILITIES-BASED STUDY

Rule Budodo[1], Dativa Pereus[2], Catherine Bakari[1], Salehe S. Mandai[1], Angelina J. Kisambale[1], Rashid A. Madebe[3], Gervas Chacha[3], Hussein J. Semboja[1], Happyness A. Mwailunga[1], Laurencia S. Tangawizi[1], Gideon I. Maulidi[1], Baraka W. Mbuba[4], Ninja S. Bisaga[3], Daniel P. Challe[5], Daniel A. Petro[6], Daniel Mbwambo[7], Sijenunu Aaron[7], Abdallah Lusasi[7], Samwel Lazaro[7], Stella Kajange[8], Jonathan J. Juliano[9], Jeffrey A. Bailey[10], Celine I. Mandara[3], Misago D. Seth[3], Deus S. Ishengoma[1]

[1]Ifakara Health Institute, Dar es Salaam, United Republic of Tanzania, [2]Muhimbili University of Health and Allied Sciences, Dar es Salaam, United Republic of Tanzania, [3]National Institute for Medical Research, Dar es Salaam, United Republic of Tanzania, [4]Nelson Mandela African Institution of Science and Technology, Arusha, United Republic of Tanzania, [5]National Institute for Medical Research, Tanga Research Centre, Tanga, United Republic of Tanzania, [6]University of Dar es Salaam, Dar es Salaam, United Republic of Tanzania, [7]National Malaria Control Programme, Dodoma, United Republic of Tanzania, [8]Prime Minister's Office Regional Administration and Local Government, Dodoma, United Republic of Tanzania, [9]Division of Infectious Diseases, University of North Carolina School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States, [10]Department of Pathology and Laboratory Medicine, Brown University, Providence, RI, United States

Malaria case management in Tanzania relies on artemisinin-based combination therapy as the first-line treatment for uncomplicated falciparum malaria and on sulfadoxine-pyrimethamine (SP) for intermittent preventive treatment in pregnancy (IPTp), making surveillance of antimalarial drug resistance markers essential. In 2022, artemisinin partial resistance (ART-R) was confirmed in Tanzania with high prevalence in Kagera, but the level of mutations associated with ART-R in other regions has not been fully established. This study determined the prevalence of markers associated with ART-R and other antimalarials across all 26 regions of Mainland Tanzania, using 10,471 positive rapid diagnostic test samples collected in 2023. The samples were sequenced on Illumina MiSeq to detect mutations in *Plasmodium falciparum pfk13*, *pfdhfr*, *pfdhps*, *pfcrt*, and *pfmdr1* genes. The *pfk13* R561H mutation was detected at 7.8% (n=109) in Kagera, 7.9% (n=5) in Katavi, and 3.4% (n=5) in Kigoma, while R622I was observed at 4.8% (n=3) in Ruvuma, 3.5% (n=3) in Katavi, 0.9% (n=1) in Morogoro, and 0.8% (n=12) in Kagera. The other *pfk13* mutations detected were A675V, P441I, and P574L at lower prevalences (less than 1.1%). The SP resistance markers (N51I, C59R, S108N, A437G, and K540E) were near-fixation across most regions (>80%), while I164L, S436A, and A581G were present at lower but variable frequencies, except in the Tanga region, where A581G was 53.8% (n=128). The mutations in *pfcrt* gene (K76T, M74I, N75E, A220S, Q271E, N326S, and R371I) were observed at higher prevalences in Arusha (>50%) than in other regions (<12%). In the *pfmdr1* gene, Y184F was the most frequent mutation with a wide distribution across all regions (39.1% to 80.6%). Overall, these findings indicate a sustained low prevalence and the spread of mutations associated with ART-R markers to more regions of Mainland Tanzania. However, the high prevalence of SP resistance mutations suggests reduced effectiveness of SP, particularly for IPTp, and underscores the need for continued molecular surveillance to inform malaria treatment and prevention policies.

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USING ROUTINE MALARIA DATA TO DESIGN WHO-ALIGNED MULTIPLE FIRST-LINE THERAPY MODELS TO PREVENT ANTIMALARIAL DRUG RESISTANCE IN NIGERIA: EVIDENCE FROM ENUGU AND KWARA STATES

Charity Ifeyinwa Anoke[1], Bright C. C. Orji[2], Olugbenga Ayodeji Mokuolu[3], Martin Meremikwu[4], Nnenna Ogbulafor[5], Emmanuel David Shekarau[6], Simon Ijezie[7], Charles E. Nzelu[8], Mohammed Abdulkadir[9], Adetiloye Oniyire[1], George Ugwu[10], Ifeyinwa Ani-Osheku[11], Chibu Meremikwu[12], Anthony Okoro[12], Oluwatosin Fakayode[13], Uchechukwu Okenwa[10], Lolade Oseni[14], Mark Kabue[15], Lara Latifat[16], Ifeoma Otiji[17], Kolawole Ajayi[18], Joseph Enne[1], Mubarak Badmasi[1]

[1]Jhpiego, Abuja, Nigeria, [2]Jhpiego, Wuye, Abuja, Nigeria, [3]University Of Ilorin, Ilorin, KW, Nigeria, [4]Univ of Calabar Teaching Hosp, Calabar, Cross River State, Nigeria, [5]Management Sciences for Health, Abuja, Nigeria, [6]National Malaria Elimination Programme, Federal Ministry of Health, Abuja, Abuja, Nigeria, [7]National Malaria Elimination Programme, Abuja, Nigeria, [8]Federal Ministry of Health, Abuja, Nigeria, [9]University of Ilorin, Ilorin, Nigeria, [10]Ministry of Health, Enugu, Nigeria, [11]Primary Health Development Agency, Enugu, Nigeria, [12]University of Calabar, Calabar, Nigeria, [13]Ministry of Health, Ilorin, Nigeria, [14]Jhpiego, Baltimore, MD, United States, [15]Jhpiego, Johns Hopkins University affiliate, Baltimore, MD, United States, [16]State Malaria Elimination Programme, Ilorin, Nigeria, [17]State Malaria Elimination Programme, Enugu, Nigeria, [18]Centre for Malaria and Tropical Diseases Research, Ilorin, Nigeria

Nigeria accounts for 24% of global malaria cases and 38% of under-five deaths, underscoring its key role in the global fight. Partial artemisinin resistance and declining partner drug efficacy in Asia and parts of Africa threaten malaria control, especially if it emerges in Nigeria. Reliance on a single ACT raises selective pressure and speeding resistance. WHO recommends Multiple First-Line Therapies (MFT) using rotational or simultaneous strategies with set thresholds for duration and ACT use. However, real-world data, especially the simultaneous approach, are scarce. We analysed retrospective malaria data from facility registers (Dec 2024-Oct 2025) across six high-burden LGAs in Enugu and Kwara States, Nigeria, to inform a national MFT pilot model. Enugu State demonstrated high transmission (81% test positivity), with adults (>12 years) representing 47% of confirmed cases. ACT usage was moderately diversified: artemether-lumefantrine (AL) 54%, dihydroartemisinin-piperaquine (DP) 19%, artesunate-amodiaquine (AA) 15%, and artesunate-pyronaridine (ASPY) 12%. Age distribution for ACT treatments in Enugu was children under five (44%), ages 5-12 years (8%), adults over 12 (44%), and pregnant women (4%). In contrast, Kwara exhibited 70% positivity and highly concentrated treatment patterns: AL accounted for 98.6% of ACT use, with other ACTs each below 1%. The age-based ACT treatment distribution in Kwara was under five (21%), 5-12 years (33%), over 12 (31%), and pregnant women (14%). These findings informed the design of Nigeria's WHO-aligned MFT pilot, which adopted an age-segmented ACT allocation: AL for children under five and pregnant women, ASPY for ages 5-12 years, and DP for individuals over 12 years. This approach aims to optimize drug distribution and target the same parasite population, promoting rational ACT diversification and long-term resistance prevention. The study demonstrates the utility of routine facility data in guiding national antimalarial stewardship and policy decisions, supporting efficient allocation of therapies and sustainable malaria control.

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IMPACT OF *PLASMODIUM FALCIPARUM* RESISTANCE-ASSOCIATED GENOTYPES ON MALARIA PARASITE CLEARANCE AND PROTECTION FROM INFECTION: A THREE-ARM DOUBLE-BLINDED, PLACEBO-CONTROLLED, RANDOMISED TRIAL OF SULFADOXINE-PYRIMETHAMINE AND SULFADOXINE-PYRIMETHAMINE PLUS AMODIAQUINE AMONG ASYMPTOMATIC CHILDREN 3-5 YEARS OLD IN CAMEROON

Andria Mousa[1], Rosario Martinez-Vega[2], Innocent M. Ali[3], Akindeh M. Nji[4], Ana Chopo-Pizarro[5], Helle Hansson[6], Miriam Niba[4], Carine Gije Nfor[4], Louise Wellmann[6], Emma Filtenborg Hocke[6], Harparkash Kaur[2], Mercy Tah-Monunde[4], Peter Thelma Ngwa Niba[4], Albertine Lele[4], Yvan F. Asaah[4], Ericka Claude Ngope[4], Stephanie Eyombo Elando[4], Charlotte Eddis[7], Lucy C. Okell[1], Roly Gosling[2], Khalid B. Beshir[2], Michael Alifrangis[6], Cally Roper[2], Colin Sutherland[2], Wilfred Fon Mbacham[8], R. Matthew Chico[2]

[1]MRC Centre for Global Infectious Disease Analysis, Department of Infectious Disease Epidemiology, Imperial College London, London, United Kingdom, [2]Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, United Kingdom, [3]Department of Biochemistry, Faculty of Science, University of Dschang, Dschang, Cameroon, [4]The Fobang Institutes for Innovation in Science and Technology, Yaoundé, Cameroon, [5]ISGlobal, Barcelona, Spain, [6]Centre for translational Medicine and Parasitology, Department of Immunology and Microbiology, University of Copenhagen, and Department of Infectious Diseases, Copenhagen University Hospital, Copenhagen, Denmark, [7]Population Services International, Washington, DC, United States, [8]The Fobang Institutes for Innovation in Science and Technology, Yaounde, Cameroon

Implementation of perennial malaria chemoprevention (PMC) with sulfadoxine-pyrimethamine (SP) is increasing across sub-Saharan Africa, but resistance-associated *Plasmodium falciparum* genotypes may compromise effectiveness. In West and Central Africa, the *pfdhps* I431V mutation is an emerging concern, but there is no *in vivo* evidence of its impact on SP clearance and protective efficacy (PE). We conducted a three-arm, double-blind, placebo-controlled randomised trial among asymptomatic children 3-5 years old in western Cameroon. Children received SP (n=449), SP plus amodiaquine (SPAQ; n=230), or artesunate control (n=223), and were followed to Day 28 by microscopy and Day 63 by qPCR at regular intervals. Clearance among Day 0 positives and 28-day PE among Day 0 negatives were analysed using Kaplan-Meier and Cox models. No significant differences in Day 28 clearance were observed between arms (qPCR: 83.3% SP vs 95.2% SPAQ, p=0.291; microscopy: 83.1% vs 83.9%, p=0.571) or by genotype. Sustained clearance to Day 28 was lower, with differences by arm (qPCR: 20.7% SP vs 66.7% SPAQ, p<0.001; microscopy: 32.6% SP vs 92.3% SPAQ, p<0.001). A significant genotype effect in sustained Day 28 clearance was observed only for microscopy in the SP arm (mutant 431V vs wildtype 431I: 22.9% vs 70.0%; p=0.009). Molecular correction will be applied and presented. Among participants without symptomatic malaria on Day 0, 28-day PE against symptomatic malaria was 40.1% (95% CI: 4.5-65.0; p=0.033) for SP and 92.9% (72.0-98.4; p<0.001) for SPAQ (SP vs SPAQ: p=0.003). By genotype, PE against symptomatic malaria did not significantly differ for SP (20.3% vs 62.9%, interaction p=0.135) or SPAQ (p=0.316). For parasitaemia outcomes, SP PE was lower against 431V than 431I by microscopy (-53.2% vs 56.2%, p=0.007) and qPCR (-10.6% vs 51.6%, p=0.033), whereas for SPAQ we saw no genotype effect by qPCR (p=0.228) and some evidence of lower PE against 431V by microscopy (p=0.035). The *pfdhps* 431V genotype was associated with reduced SP efficacy against parasitaemia, providing the first *in vivo* evidence that this emerging mutation compromises SP-based chemoprevention.

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MALARIA RESURGENCE IN RWANDA: SELECTION ON STANDING *PLASMODIUM FALCIPARUM* DIVERSITY AND EMERGENCE OF A COMPOSITE *KELCH13* RESISTANCE SIGNATURE

Mariama Telly Diallo[1], Edgar Kalimba[1], Sandra Fankem Noukimi[1], Jean-Bosco Mbonimpa[2], Aimee Nadine Nsengiyumva Ishimwe[2], Aloysie Basoma Biryuwenze[2], Arsene Musana Habimana[2], Louis de Mont Fort Ntwali Mugisha[2], Mary Teke Efeti[3], Irene Molina de la Fuente[4], Anna Rosanas-Urgell[4], Jacob Souopgui[1]

[1]Université Libre de Bruxelles, Charleroi, Belgium, [2]Rwanda Malaria Research Lab, Kigali, Rwanda, [3]Vrije Universiteit Brussel, Brussels, Belgium, [4]Institute of Tropical Medicine, Antwerp, Belgium

After more than a decade of sustained decline, Malaria resurged in Rwanda in 2024, reversing earlier public health gains and raising concerns about what is driving this shift and what it means for malaria control in sub-Saharan Africa. A central question was whether this resurgence was driven by the introduction of new parasite populations or by evolutionary changes within local populations. To explore this, we analysed 1179 *Plasmodium falciparum* isolates collected between 2021 and 2025 from urban and high-transmission settings across Rwanda. We combined targeted sequencing of key drug-resistance genes (*kelch13, crt, mdr1, dhfr,* and *dhps*) with population genetic analyses using the polymorphic *csp* and *msp2* markers. Temporal and spatial patterns were examined using principal component and phylogenetic approaches, while selected *kelch13* genotypes were tested *in vitro* through ring survival and fitness assays to better understand their behaviour. We did not observe evidence of parasite population replacement. Instead, the data point to an expansion of pre-existing genetic diversity. One pattern stood out: parasites carrying the *kelch13* R561H-K189T genotype increased markedly over time, particularly in Kigali. R561H is already recognised as a marker of partial artemisinin resistance, whereas K189T has generally been considered a background variant. In our assays, parasites with both mutations consistently outcompeted others in mixed infections, suggesting a possible fitness advantage that could help explain their spread. We also identified a small number of isolates carrying multiple resistance-associated mutations across different loci, consistent with multidrug-resistant backgrounds. These remain uncommon, but their presence suggests a reservoir that could expand under continued drug pressure. Overall, our findings indicate that malaria resurgence in Rwanda is more likely driven by selection acting on existing parasite diversity than by the introduction of new lineages, and they reinforce the need to combine genomic surveillance with functional approaches to better anticipate emerging resistance in African settings.

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NOVEL AFRICAN *PFK13* BTB/POZ-DOMAIN MUTATION CONFERS ARTEMISININ TOLERANCE IN *PLASMODIUM FALCIPARUM*

Bruno José Ferreira Freitas[1], Maria Eduarda Pereira Mascarenhas[2], Nuno Osório[1], Romain Coppée[3], Maria Isabel Veiga[1]

[1]Life and Health Sciences Research Institute, Braga, Portugal, [2]Instituto René Rachou/Fiocruz Minas, Belo Horizonte, MG, Brasil, Minas Gerais, Brazil, [3]Université Rouen Normandie, Normandie, France

The emergence of artemisinin tolerance in African parasite populations, causers of the highest global burden of Malaria, poses a major threat to malaria control efforts. Artemisinin tolerance in *Plasmodium falciparum* is clinically defined by delayed parasite clearance following an artemisinin-based combination therapy and is primarily associated with non-synonymous mutations in the parasite's *pfk13* gene encoding the Kelch13 protein. To date, all World Health Organization–validated tolerance-associated mutations map to the protein's Kelch propeller domain, with the functional contribution of non-Kelch domain variants remaining poorly understood.In a case of severe malaria imported from Mozambique, we identified a single-nucleotide polymorphism, I416V, within the BTB/POZ domain of *pfk13*. Using CRISPR–Cas9 genome editing, we generated

an isogenic mutant parasite line which displayed an increased survival (~3.35%) following exposure to the clinical dose of dihydroartemisinin. Structural simulations further indicate that this mutation disrupts a key contact between the BTB and Propeller domains, likely leading to altered protein behavior, which may underlie the observed phenotype. Moreover, in light of prior evidence that *pfk13* mutations influence the parasite life cycle, we examined the intraerythrocytic cycle dynamics of our mutant line over 48 hours and observed a shortened cycle duration, characterized by an earlier commitment to replication.Together, these findings validate a novel K13 mutation conferring reduced artemisinin susceptibility and identify a previously underrecognized structural determinant of artemisinin tolerance outside the canonical Kelch domain. Our results highlight the potential clinical relevance of BTB/POZ-domain variants circulating in African parasite populations and support their consideration in molecular surveillance efforts aimed at monitoring the emergence and spread of artemisinin tolerance.

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MARKER CHOICE AND ANALYTICAL METHOD ALTER RECRUDESCENCE ESTIMATES IN A REANALYSIS OF UGANDAN TREATMENT FAILURES

Jessica Briggs[1], Giselle Lima-Cooper[2], Ruth Namazzi[3], Ryan Henrici[4], Colin Sutherland[5], Robert Opika Opoka[6], Inna Gerlovina[1], Chandy C. John[7], Bryan Greenhouse[8], Melissa Conrad[9]

[1]UCSF, San Francisco, CA, United States, [2]Indiana University School of Medicine, Indianapolis, IN, United States, [3]Makerere University, Kampala, Uganda, [4]University of Pennsylvania, Philadelphia, PA, United States, [5]London School of Hygiene & Tropical Medicine, London, United Kingdom, [6]College of Health Sciences, Makerere Univ, Kampala, Uganda, [7]Indiana University School of Medicine, Saint Paul, MN, United States, [8]Univ of California San Francisco, San Francisco, CA, United States, [9]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

Estimates of *Plasmodium falciparum* (Pf) recrudescence rates may vary according to genotyping method. In a study evaluating Pf artemisinin partial resistance (ART-R), Henrici et al demonstrated a high rate of ART-R (11%) and of 28-day recrudescence after treatment with parenteral artesunate and oral ACT. Of 97 children tested, 11 had recurrent parasitemia; MSP-2 genotyping classified 10 of these 11 recurrences as recrudescent (PCR-adjusted recrudescence rate 10.3%). However, ART-R was not related to 28-day recrudescence. The World Health Organization recommends the characterization of 3 length polymorphism loci for evaluation of recrudescence, and in high transmission settings such as Uganda, even 3 diverse loci can have limited discriminatory power. Therefore, we performed additional genotyping on all 11 paired samples. MSP1 and GLURP were genotyped to complement the original MSP-2 data and analyzed using 2/3 and 3/3 match-counting algorithms. Microsatellite genotyping was attempted but failed. Samples were also characterized using the MAD^{4}HatTeR (MH) amplicon sequencing panel (165 diverse microhaplotypes), with outcomes classified by Aster, which accounts for chance matching of alleles, population allele frequencies and undetected minority clones. The number of recurrences classified as recrudescent were 7, 4, and 3 under 2/3, 3/3, and MH/Aster, yielding PCR-adjusted 28-day recrudescence rates of 6.3%, 4.1%, and 3.1%. Despite similar totals, 3/3 and MH/Aster agreed on only 8 of 11 pairs; in two cases both traditional methods called recrudescence while MH/Aster classified the recurrence as reinfection, suggesting that even when traditional markers agree, microhaplotype-based resolution can detect sufficient allelic divergence to reclassify the outcome. In one case, 2/3 and MH/Aster agreed on recrudescence while 3/3 did not. ART-R was not associated with recrudescence in any of the classifications. These findings demonstrate that recrudescence classification is highly sensitive to marker choice and analytical method, with implications for accurately estimating recrudescence rates in high-transmission settings.

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SAFETY OF PYRONARIDINE-ARTESUNATE IN THE FIRST TRIMESTER OF PREGNANCY: FINDINGS FROM THE MIMBA PREGNANCY EXPOSURE REGISTRY

Benard Omondi[1], Toussaint Rouamba[2], Hellen Cherono Barsosio[1], Mercy Chepkurui Terer[1], Athanase Mwinessobaonfou Some[2], Kennedy Maube[1], Michael Alaw[1], Benard Asuke Opiyo[1], Caroline Osoro[3], Martina Oneko[1], Innocent Valea[2], Simon Kariuki[1], Aninda Nishat Moitry[4], Rhys Peploe[4], James Watson[4], Anton Kalyuzhnyy[5], Myriam El Gaaloul[6], Stephan Duparc[6], Jenny Hill[5], Phillipe J. Guerin[4], Wiweka Kaszubska[6], Halidou Tinto[2], Feiko Ter Kuile[5], Stephanie Dellicour[5]

[1]Kenya Medical Research Institute, Centre for Global Health Research, Kisumu, Kenya, [2]Clinical Research Unit of Nanoro, Nanoro, Burkina Faso, [3]KEMRI-Wellcome Trust Research Program, Nairobi, Kenya, [4]Infectious Diseases Data Observatory, University of Oxford, Oxford, United Kingdom, [5]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [6]Medicines for Malaria Venture (MMV), Geneva, Switzerland

Evidence on the safety of artemisinin-based combination therapies (ACTs) in early pregnancy remains limited, despite increasing diversity of ACT use. Artemether-lumefantrine (AL) is the WHO-recommended treatment for uncomplicated *Plasmodium falciparum* malaria in the first trimester, having the most safety data among ACTs for this population. Pyronaridine-artesunate (PA) is increasingly deployed across sub-Saharan Africa but lacks human safety data in the first-trimester. The MiMBa Pregnancy Exposure Registry, a prospective, observational cohort study, collected evidence on the safety of a range of ACTs during pregnancy. It enrolled women of childbearing age and pregnant women from community and health facility settings in Kenya and Burkina Faso. Data on illnesses and drug exposures were captured prospectively through health facility records and structured interviews. The primary outcome was a composite adverse pregnancy outcome comprising spontaneous pregnancy loss and major congenital anomalies, ascertained at delivery and through infant follow-up to one year, with standardised screening for congenital heart defects. Confirmed AL- and quinine (QN)-exposed pregnancies served as reference groups. Among 15,391 pregnancies, 786 had first-trimester antimalarial treatment, including 127 confirmed exposures to AL, 46 to PA and 134 to QN. The composite safety endpoint occurred in 10/127 (7·9%) AL-exposed, 3/46 (6·5%) PA-exposed, and 11/134 (8·2%) QN-exposed pregnancies. No major congenital anomalies were observed among infants exposed in-utero to AL or PA, while two congenital heart defects were recorded among QN-exposed infants. Results of comparative analyses using flexible parametric models will be presented at the meeting. These preliminary findings provide the first human safety data on PA use in the first trimester of pregnancy and are reassuring, with no indication of excess adverse pregnancy outcomes for PA compared with AL. While limited by sample size, these data will contribute to the evidence base needed to inform risk-benefit assessments as treatment strategies evolve for women of childbearing potential.

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HIGH RATES OF ANTIMALARIAL OVERTREATMENT IN PRIVATE PHARMACIES IN BENIN: EVIDENCE FROM A NATIONALLY REPRESENTATIVE STUDY OF PHARMACY CLIENTS

Paul Bouanchaud[1], Cyprien Zinsou[2], Isidore Kikissagbe[3], Ghyslain Guedegbe[3], Manfred Mario Kokou Accrombessi[4]

[1]PSI, Washington, DC, United States, [2]PSI/ABMS, COTONOU, Benin, [3]ABMS, Cotonou, Benin, [4]Cotonou, Benin

Presumptive antimalarial use remains common in malariaendemic settings despite WHO recommendations for parasiteconfirmed treatment. In Benin, only 41% of febrile children seeking care attend public facilities, indicating substantial reliance on the private sector. Antimalarial overtreatment increases household costs, delays management of nonmalarial illness, and sustains drug pressure that may accelerate resistance. A 2023 ACTwatch Lite study showed private pharmacies dominate antimalarial

distribution in Benin but do not provide diagnostics. We conducted a nationally representative crosssectional study among clients purchasing antimalarials in 136 randomly selected private pharmacies during the rainy season (Oct-Nov 2025). Consenting participants received malaria RDTs (SD BIOLINE™ Malaria Ag Pf, WHOPQ). Overtreatment was defined as antimalarial purchase without confirmed malaria (negative RDT or no prior positive test). Analyses were weighted for sampling design, and multivariable logistic regression identified factors associated with overtreatment. Pharmacybased sampling was skewed toward southern and urban areas. Among 864 clients, 96.6% accepted RDT testing. Malaria positivity was 12.8% (adjusted to include participants reporting prior results), indicating that nearly nine in ten antimalarial purchases occurred without confirmed malaria. Overtreatment was significantly more common in southern and urban regions and among clients with higher education and household socioeconomic status. Prior malaria testing reduced likelihood of overtreatment, yet testing uptake before pharmacy visits remained rare (<11%). In conclusion, malaria overtreatment is widespread in Benin's private pharmacies despite high ACT adherence and strong RDT acceptance when offered. Scaling affordable, routine pharmacybased malaria testing, integrated with privatesector surveillance and marketintelligence systems, could reduce inappropriate ACT use, improve fever management, and mitigate antimalarial drug resistance.

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EXPANDING ACCESS OR EMERGING RISK? USING MAISHA ACT TO IDENTIFY A TEN-FOLD SURGE IN LOW-COST INJECTABLE ARTESUNATE USE IN THE AFRICAN PRIVATE SECTOR

Alex C. Kong, Adriana M. Sousa, Natasha Mathur, Florence Muriuki, Daniel L. Rosen

Maisha Meds, San Francisco, CA, United States

Injectable artesunate is the World Health Organization's first-line treatment for severe malaria, followed by a course of antimalarial-containing combination therapies (ACTs) to prevent the development of artemisinin resistance. Despite clear guidance from the WHO reserving use of this drug for severe malaria, its use as a monotherapy for uncomplicated malaria is a growing concern. We used Maisha ACT (Antimalarial Consumption Tracker) to analyze consumption and price trends from 2017-2025. Maisha ACT tracks ~90% of African antimalarial consumption by integrating international trade data, donor records, and last-mile facility data across the public and private sectors. From 2017-2025, the volume of injectable artesunate imported to Africa increased ten-fold from 19M to 191M vials. This was driven by a 3,230% increase in the private sector compared to 52% in the public sector. By 2025, the private sector accounted for 89% of all monitored vials. We observed a shift in product strength preference: the proportion of 120 mg vials in the private sector doubled from 19% (2017) to ~39% from 2023-2025, while the public sector consistently prioritized 60 mg vials (87-99%). Over the period of analysis, the average price per vial of WHO-prequalified 60 mg artesunate procured by the public sector fell just 10% to $1.29 in 2025: five times the price the private sector paid for non-prequalified 60 mg artesunate and four times that of non-prequalified 120 mg artesunate. Country-specific analyses showed differing upward trends of injectable artesunate consumption with the most pronounced increase for South Sudan, pentupling between 2024 and 2025. The rapid expansion of low-cost injectable artesunate in Africa (2017-2025) without a commensurate increase in ACT volumes, suggests widespread use as a monotherapy, misaligned with WHO clinical guidance. This pattern of sub-therapeutic exposure, coupled with the unknown quality of non-prequalified vials, represents a critical and under-monitored driver of artemisinin resistance in sub-Saharan Africa.

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IMPLEMENTING ROTATIONAL MULTI-FIRST-LINE THERAPY TO PRESERVE ACT EFFICACY - EARLY LESSONS FROM THREE HEALTH ZONES IN THE DEMOCRATIC REPUBLIC OF CONGO

Deogratias Cibinda Ntale[1], Alain Mikatho[1], Basimike Mulenda[2]

[1]Jhpiego, Kinshasa, Democratic Republic of the Congo, [2]Consultant, Kinshasa, Democratic Republic of the Congo

The Democratic Republic of Congo (DRC) bears one of the world's highest malaria burdens, with sustained pressure on a small number of artemisinin-based combination therapies (ACTs). Such reliance heightens the long-term risk of *Plamodium falciparum* resistance in hightransmission settings. Multi First Line Therapy (MFT) approach, which rotates ACTs to diversify treatment pressure, offers a promising pathway to preserve ACT efficacy. The STOPAMDR Project (2025-2028) is piloting a rotational MFT model in three DRC health zones, Kingasani (urban), Kenge and Vanga (rural) to assess its feasibility, acceptability, and operational readiness for national scaleup. Pre-pilot activities already conducted are national consensus-building on the MFT model, establishment of a multisectoral MFT coordination mechanism, and commodity supply. The MFT pilot introductory phase (Apr-Nov 2026) activities includes ACT redeployment (replacement of ASAQ with AL in rural, and in urban AL with ASAQ); provider training; guideline revision; prospective ACT quantification; enhanced routine data monitoring (DHIS2); pharmacovigilance; private-sector engagement; and supportive supervision. Early process indicators focused on stakeholder adherence to MFT, facility readiness, supply continuity, and provider compliance with ACT assignment. This introductory phase is expected to demonstrate strong feasibility and early adoption of the MFT model across the pilot zones. Anticipated outcomes include ≥85% adherence to assigned ACTs, >95% of health facilities (HF) maintaining ACT stock and improving provider compliance. Additional findings will capture the production of highquality HF data on MFT implementation, including privatesector and community participation, health system readiness, barriers and facilitators, and stakeholders' capacity to coordinate the MFT within the 2025-2030 resistancemitigation plan. Early insights will guide realtime adjustments to optimize the 24month MFT rollout, while the DRC's model will help shape national malaria policy and global strategies to sustain ACT efficacy and prevent resistance.

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TEMPORAL TRENDS AND CHARACTERISTICS OF *PLASMODIUM. FALCIPARUM* ANTIMALARIAL DRUG RESISTANCE MARKERS *MDR1* 86Y AND *CRT* 76T IN KINSHASA PROVINCE, DEMOCRATIC REPUBLIC OF THE CONGO

Ruthly François-Zafka[1], Abebe A. Fola[2], Melchior Mwandagalirwa Kashamuka[3], Rebecca Crudale[2], Kristin Banek[1], Samuel J. White[1], Joseph A. Bala[3], Marthe Nkalani[3], Georges Kihuma[3], Joseph A. Losoma[3], Georges E. Mahilu[3], Kyaw L. Thwai[1], Maxwell Igwe[1], Elvira Diouf[1], Rhoel R. Dinglasan[4], Alfred Simkin[2], Michael Emch[1], Jessie K. Edwards[1], Daniel Westreich[1], Jonathan J. Juliano[1], Jeffrey A. Bailey[2], Antoinette Tshefu Kitoto[3], Jonathan Parr[1]

[1]University of North Carolina at Chapel Hill, Chapel Hill, NC, United States, [2]Brown University, Providence, RI, United States, [3]University of Kinshasa, Kinshasa, Democratic Republic of the Congo, [4]University of Florida, Gainesville, FL, United States

Increasing reports of artemisinin partial resistance and decreasing partner drug sensitivity in Africa raise concerns about the long-term efficacy of artemisinin-based combination therapies (ACTs) for *Plasmodium falciparum* malaria. We studied temporal trends and characteristics of polymorphisms at the *mdr1* N86Y and *crt* K76T loci associated with reduced susceptibility to lumefantrine and amodiaquine, the partner drugs used in first-line ACTs in the Democratic Republic of the Congo (DRC). Using molecular inversion probe sequencing, we genotyped drug-resistance loci in *P. falciparum*

samples collected from children and adults enrolled in longitudinal malaria cohort studies in Kinshasa Province, DRC, between 2015 and 2022. We calculated annual prevalence of *mdr1* 86Y and *crt* 76T and used Bayesian mixed-effects linear models to estimate their selection coefficients (denoted *s*) and generalized estimating equations to assess associations between these markers and the introduction of artemether-lumefantrine (AL) in 2019, as well as clinical and demographic factors. The prevalence of *mdr1* 86Y declined from 33.0% (95% CI: 28.0, 38.0%) in 2015 to 16.4% (95% CI: 13.3, 19.6%) in 2021-2022 at rural and peri-urban sites, consistent with negative selection [*s* = -0.19 (95% Bayesian Credible Interval, BCrI: -0.29, -0.08)]. The prevalence of *crt* 76T also declined from 74.0% (95% CI: 69.4, 78.6%) in 2015 to 43.2% (95% CI: 39.1, 47.3%) in 2021-2022 across sites, with stronger negative selection [*s* = -0.31 (95% BCrI: -0.36, -0.27)]. Infections with *mdr1* 86Y showed modestly higher parasite densities and odds of fever than N86 infections, though differences were not significant. While parasite densities were similar, *crt* 76T infections were less likely to be symptomatic [OR for fever = 0.83 (95% CI: 0.72, 0.96)]. The prevalence of both markers declined with age, urbanicity, and following AL introduction. Both *crt* 76T and *mdr1* 86Y declined over time in Kinshasa, consistent with negative selection following ACT policy changes. Continued molecular surveillance is critical to monitor evolving resistance patterns and inform malaria treatment policies.

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REASSESSING ANTIMALARIAL POTENCY IN MALI: INSIGHTS FROM MULTISITE *EX VIVO* SUSCEPTIBILITY TESTING

Fatoumata Ousmane Maiga, Laurent Dembele, Abdoulaye Djimde

University of Sciences, Techniques and Technology of Bamako (USTTB), Faculty of Pharmacy, Parasites and Microbes Research and Training Center (PMRTC), Point G. PBE: 1805, Bamako, Mali

Malaria remains a major public health burden in Africa, where evolving *Plasmodium falciparum* resistance threatens the effectiveness of current therapies. Continuous monitoring of drug susceptibility is essential to inform treatment strategies, especially as older drugs show changing activity profiles and new candidates progress toward clinical deployment. This study assesses the *ex vivo* efficacy of a broad panel of antimalarials, withdrawn, currently used, and nextgeneration, across three ecological sites in Mali. *P. falciparum* isolates (n=26) were collected from Faladje, Kolle, and BougoulaHameau and tested using SYBR Green I and Mitotrackerbased assays. Fourteen compounds (14) were evaluated, including chloroquine, amodiaquine, dihydroartemisinin, lumefantrine, pyronaridine, quinine, sulfadoxine, pyrimethamine, atovaquone, cabamiquine, KDU691, and GNF179. IC_{50} values were derived by flowcytometry-based doseresponse analysis. antimalarials such as dihydroartemisinin, lumefantrine, pyronaridine, amodiaquine, and atovaquone retained strong activity across sites. In contrast, sulfadoxine and pyrimethamine showed markedly elevated IC_{50} values, exceeding 1,000 nM, indicating substantial loss of efficacy. Chloroquine and quinine demonstrated considerable intersite variability, reflecting heterogeneous resistance patterns. Nextgeneration compounds, GNF179, cabamiquine, and KDU691, exhibited high potency, with GNF179 and cabamiquine maintaining IC_{50} <10 nM, confirming their suitability for future development. In conclusion, This multisite evaluation highlights the continued strength of ACT partner drugs but also documents significant resistance to antifolates and variable susceptibility to older therapies. The robust performance of nextgeneration agents supports their advancement and underscores the need for ongoing surveillance to guide treatment policy and optimise future drug deployment in Mali.

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GENOTYPE-PHENOTYPE ASSOCIATIONS FOR STANDARD AND NEW ANTIMALARIAL COMPOUNDS AGAINST *PLASMODIUM FALCIPARUM* ISOLATES FROM BURKINA FASO

Oriana Kreutzfeld[1], A. Fabrice SOME[2], Thomas Bazié[2], Zachari Kabré[2], Aminata Fofana[2], Serge Yerbanga[2], Jenny Legac[1], Frederic Wendlamita Nikiema[3], Anna Adam[4], Jeffrey A. Bailey[5], Roland A. Cooper[6], Philip Rosenthal[1]

[1]University of California San Francisco, San Francisco, CA, United States, [2]Institut de Recherche en Sciences de la Santé, Direction Régionale de l'Ouest, Bobo-Dioulasso, Burkina Faso, [3]Institut de Recherche en Sciences de la Santé, Direction Régionale de l'Ouest, Bobo Dioulasso, Burkina Faso, [4]Medicines for Malaria Venture, Geneva, Switzerland, [5]Brown University, Providence, RI, United States, [6]Dominican University of California, San Rafael, CA, United States

Increasing resistance to frontline antimalarials underscores the need to develop novel antimalarials. To characterize activities against parasites circulating in Africa, we assessed ex vivo susceptibilities of 452 *Plasmodium falciparum* clinical isolates collected in Burkina Faso from 2021 to 2025 to 10 standard antimalarials and 23 test compounds at varied stages of development. Susceptibilities were determined by half-maximum inhibitory concentrations (IC_{50}s) from dose response curves using a 72 h SYBR-green assay. Susceptibilities to standard antimalarials were excellent (median IC_{50}s (nM): chloroquine 9.8, monodesethylamodiaquine 17.2, piperaquine 6.2, pyronaridine 1.7, lumefantrine 7.5, mefloquine 7.8, dihydroartemisinin 2.4, quinine 49.6). Field isolates were also generally highly susceptible to test compounds, with low-mid-nM median IC_{50}s. Isolates were sequenced for mutations in 80 genes encoding potential resistance mediators. We identified low prevalence of aminoquinoline resistance mutations and high prevalence of antifolate resistance mutations. We saw several polymorphisms in potential drug targets and transporters, but most were not associated with altered compound susceptibilities. The P-type cation translocating ATPase (PfATP4) G223S mutation (seen in 28.9%) was associated with modestly decreased susceptibility to 3 PfATP4 inhibitors (mean IC_{50}, nM: SJ733 56.4 WT, 74.8 mutant; PA92 7.9 WT, 13.6 mutant; cipargamin 0.88 WT, 1.2 mutant; $p<0.001$ for all comparisons). Decreased susceptibility of mutant parasites to these PfATP4 inhibitors was previously observed in Ugandan isolates; a different substitution at the same codon (G223R) selected in vitro was associated with a modest decrease in PfATP4 inhibitor susceptibility. We did not identify other resistance mutations previously selected in vitro, including with ganaplacide and M5171. Overall, malaria parasites circulating in Burkina Faso were generally susceptible to standard antimalarials and 23 compounds now under development, with modest changes in susceptibilities to PfATP4 inhibitors associated with a mutation in PfATP4.

7120

COMPARATIVE DRUG STUDY ON THE RELATIVE EFFICACIES OF CHLOROQUINE AND ARTEMISININ DERIVATIVES IN THE TREATMENT OF CHILDREN INFECTED WITH MALARIA

Andrea T. Kassim[1], Ousmane Koita[2], Aliou Sissako[3]

[1]New Beginnings Institute, LLC, Roanoke, VA, United States, [2]University of Science Techniques and Technologies of Bamako, Bamako, United States, [3]Univ of Bamako, Bamako, Mali

The Laboratoire de Biologie Moléculaire Appliquée (LBMA) of the University of Bamako followed a cohort of over 300 children in the village of Missira, Mali for a period of two months and identified those children infected with the malaria parasite by physical examination and laboratory blood tests. Splenomegaly was used as the clinical parameter to screen children for malaria infection, after which the children's blood samples were examined for malaria parasitemia. Twenty children were identified as having malaria parasitemia and were subsequently divided into two groups. The first group of children received chloroquine as treatment and the second group received the artemisinin-based combination therapy dihydroartemisinin/

piperaquine as malaria treatment. After administration of all the required doses of each respective medication, the children were followed for a period of ten days. During this time, the children's recovery was ascertained by direct observation of clinical symptoms and microscopic analysis of blood smears. The children who received the chloroquine as treatment were found to have a 90% reduction in parasitemia, a 33% reduction in the incidence of splenomegaly as well as a 6% increase in hematocrit. The children who received the artemisinin-based combination therapy dihydroartemisinin/piperaquine as treatment were found to have a 100% reduction in parasitemia, a 38% reduction in the incidence of splenomegaly as well as a 5% increase in hematocrit. Based on this set of data, it can be concluded that in the village of Missira, Mali chloroquine is equally effective as artemisinin-based combination therapy dihydroartemisinin/piperaquine in the treatment of malaria parasitemia.

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IMPACT OF INTERMITTENT PREVENTIVE TREATMENT WITH DIHYDROARTEMISININ-PIPERAQUINE ON PARASITAEMIA AND HAEMOGLOBIN AMONG SCHOOLCHILDREN IN TANZANIA

Willy Le Roi Togna Pabo[1], Mahdi Safarpour[1], Vito Baraka[2], Dady M Helefu[2], Geofrey Makenga[1], Jean-Pierre Van geertruyden[1], **Christopher Delgado Ratto**[1]

[1]University of Antwerp, Antwerp, Belgium, [2]National Institute for Medical Research, Tanga, United Republic of Tanzania

Malaria remains a major public health challenge in sub-Saharan Africa, where asymptomatic school-aged children can represent up to 70% of infections and act as an important reservoir for transmission. Tanzania continues to carry a high malaria burden and uses dihydroartemisinin-piperaquine (DP) as an alternative first-line treatment, although emerging drug resistance may threaten its long-term effectiveness. This study evaluated the impact of intermittent preventive treatment with DP on parasitaemia and health outcomes among asymptomatic schoolchildren. We analyzed data from a cluster-randomized trial conducted in 2020 among 3,718 schoolchildren in Tanzania. The intervention group received DP every four months, while the control group received standard care. The primary outcome was parasitaemia at 12 months. Secondary outcomes included haemoglobin (Hb) concentration, anaemia prevalence, and the prevalence of molecular markers associated with piperaquine resistance. There was no significant difference in parasitaemia between groups at 12 months (p = 0.942). Haemoglobin concentrations increased over time in the DP group, reaching a mean of 12.43 g/dL (±1.40) compared with 12.31 g/dL (±1.51) in the control group. Anaemia prevalence decreased over the follow-up period in the intervention group. Molecular markers associated with piperaquine resistance were rare (0.2%). In multivariable analysis, children receiving DP for 12 months were more likely to be parasite-negative at 12 months (OR = 2.62, 95% CI: 1.60-4.33).Intermittent preventive treatment with DP was associated with improved haemoglobin levels and modest effects on parasite outcomes. Although no difference was observed in crude comparisons, adjusted analyses suggested an association between DP exposure and parasite negativity. No evidence of widespread piperaquine resistance was observed. These findings support the use of DP as part of school-based malaria control strategies in high-transmission settings.

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RECURRENT *PLASMODIUM MALARIAE* INFECTIONS IN THE DEMOCRATIC REPUBLIC OF THE CONGO

Wenqiao He[1], Rachel Sendor[2], Melchior Kashamuka[3], Kristin Banek[4], Zachary Ray Popkin-Hall[5], Jonathan J. Juliano[6], Antoinette Tshefu Kitoto[7], Jonathan Parr[1]

[1]University of North Carolina at Chapel Hill, Chapel Hill, NC, United States, [2]University of North Carolina, Chapel Hill, Chapel Hill, NC, United States, [3]Kinshasa School of Public Health, Kinshasa, Democratic Republic of the Congo, [4]UNC Institute for Global Health & Infectious Diseases, Chapel Hill, NC, United States, [5]University of North Carolina at Chapel Hill School of Medicine, Chapel Hill, NC, United States, [6]Univ of North Carolina, Chapel Hill, NC, United States, [7]Kinshasa School of Public Health, Kinshasa, United States

Plasmodium malariae, a neglected malaria parasite associated with clinically significant symptoms, is widely distributed across African countries, where it often co-circulates with *P. falciparum.* Persistent *P. malariae* infection has been reported decades after initial confirmed infection. We developed a multilocus sequencing assay to distinguish *P. malariae* re-infection from recrudescence in a longitudinal cohort study from 2015 to 2017 in Kinshasa, DRC. Among 9,089 samples from 1,565 participants, 58 participants had recurrent *P. malariae* infections, comprising 131 *P. malariae* infections. During assay development, six microsatellite loci and partial *ama1* gene sequences were amplified and sequenced from 20 randomly selected samples using Oxford Nanopore MinION. Microsatellite data were analyzed by SeekDeep and *ama1* sequences processed with an in-house bioinformatics pipeline for base-calling, filtering, demultiplexing, trimming, alignment, and variant calling. Nanopore-derived microsatellite lengths were compared with capillary electrophoresis, showing high concordance for four loci. We further evaluated an assay including these four microsatellites and *ama1* using whole-genome sequencing data from a subset of 13 infections from five individuals, revealing higher genetic diversity across microsatellite loci and *ama1*. Applying this optimized assay to 129 *P. malariae* infections from 57 individuals enrolled in the parent study, we successfully genotyped all loci in 96 samples from 41 participants with multiple *P. malariae* infections. Data was analyzed using SeekDeep, Aster, and match-counting. Results confirmed three recrudescent infections with identical genotypes, occurring 3-68 days apart. Our assay targeting *ama1* and microsatellites successfully differentiates *P. malariae* re-infection from relapses and confirmed recrudescent infection in Kinshasa, DRC. Combining sequencing results with available clinical and demographic data, we aim to improve our understanding of recurrent *P. malariae* infections in the DRC and other endemic countries.

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PRE-SEASON ASYMPTOMATIC MALARIA INFECTION AND SUBSEQUENT RISK IN AN AREA COVERED WITH SEASONAL MALARIA CHEMOPREVENTION: EVIDENCE FROM A LONGITUDINAL COHORT STUDY IN MALI

Keita Soumba[1], Mahamoudou Touré[1], Ibrahim Sanogo[1], Daouda Sanogo[1], Fousseyni Kane[1], Hamady Coulibaly[1], Cheick Oumar Tangara[1], Jian Li[2], Jeffrey G Shaffer[2], Seydou Doumbia[1]

[1]UCRC/USTTB, Bamako, Mali, [2]Tulane University, New Orleans, LA, United States

In malaria-endemic areas of Mali, young children commonly carry asymptomatic *Plasmodium falciparum* infections that sustain transmission and increase risk of subsequent clinical disease. As Mali scaled up seasonal malaria chemoprevention (SMC), understanding persistence of pre-seasonal parasitemia and its risk for subsequent clinical malaria is critical for refining targeted control strategies. We analyzed data from repeated cross-sectional surveys and passive case detection conducted between 2014-2019 in two high-transmission villages (Dangassa and Koila Bamanan). The cohort included 508 children under 2 years, with 608 clinical episodes recorded. Asymptomatic infection at start of transmission season was detected by microscopy. Clinical malaria was defined as fever (axillary temperature > 37.5°C) with a positive rapid diagnostic test or microscopy. Kaplan-Meier survival analysis and Cox proportional hazards models (adjusted for age, sex, site, and year) were applied to assess associations between pre-season parasitemia and subsequent clinical malaria. Asymptomatic *P. falciparum* prevalence declined following SMC scale-up but remained persistent at low levels (21.3% in 2014 to 4.8% in 2019). In 2014, children infected in the pre-season experienced higher clinical malaria incidence than uninfected peers (65.0% vs. 33.8%). Severe malaria features were more frequent among pre-season-infected compared to uninfected children (32.8% vs 23.8%, p = 0.041)**.** Pre-season positive parasitemia was associated with earlier onset (log-rank p = 0.003). Crude analysis showed increased risk (HR 2.09, 95% CI 1.27-3.43, p = 0.004),

though this association attenuated after adjustment (aHR 1.51, 95 % CI 0.89-2.57). Malaria risk was higher in Dangassa than Koila Bamanan (aHR 2.70, 95% CI 1.69-4.17). Pre-season asymptomatic infection may be a determinant of children at high risk of clinical malaria, even in areas covered by SMC. Residual infections remain clinically relevant, supporting integrated strategies combining SMC with targeted surveillance of asymptomatic to further accelerate malaria control in high-burden settings.

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RISK FACTORS FOR *PLASMODIUM FALCIPARUM* INFECTION IN EARLY PREGNANCY BY HS-RDT AND QPCR IN KENYA AND DR CONGO

Jean Okitawutshu[1], Jeremiah Laktabai[2], Melissa Bauserman[3], Daja Denise Gaston[4], Fabian Omoding Esamai[5], Antoinette Tshefu Kitoto[6], Steve Myer Taylor[7]

[1]Kinshasa School of Public Health, Kinshasa, Democratic Republic of the Congo, [2]Moi University, Eldoret, Kenya, [3]University of North Carolina, Chapel Hill, NC, United States, [4]Durham, NC, United States, [5]Alliance for Maternal and Child Health, Busia, Kenya, [6]Kinshasa School of Public Health, Kinshasa, United States, [7]Duke University, Durham, NC, United States

Antenatal malaria prevention is currently suboptimal in the 1st trimester of pregnancy. Later in pregnancy, ITN use, effective case management, and IPTp-SP use mitigate the effects of *Plasmodium falciparum* infections on the pregnancy, but these interventions are impractical owing to either delayed presentation to ANC or contraindication in the 1st trimester. During this period, several lines of evidence suggest that pregnancies are uniquely vulnerable to *P. falciparum* infection, which can interfere with placentation and disrupt fetal growth trajectories. A better understanding of the risk factors for these early pregnancy infections could help to target prevention or active screening strategies to those at highest risk. We performed parasite detection by high-sensitivity rapid diagnostic test (HS-RDT) and by real-time qPCR in women in the 1st trimester as a part of a clinical trial in DR Congo and Kenya. In rural clinics at each site, women were enrolled if they had ultrasound-confirmed singleton pregnancies ≤13 6/7 weeks gestation, were HIV-uninfected, and had not received recent antimalarials. Women were randomized to either usual care or to screening with the HS-RDT (Abbott NxTek Eliminate) followed by treatment with AL if positive. Participants were followed through delivery. From dried blood spots collected at the time of HS-RDT testing, we performed parasite detection and density estimation using a highly-sensitive qPCR assay. To date, the overall parasite prevalence is 36.4% by HS-RDT and 71.7% by qPCR. Risk factors for these infections will be explored, including maternal age, gestational age, gravidity, ITN use, study site, and SES. These data will identify women at elevated risk of prevalent *P. falciparum* infection in the 1st trimester who may be efficiently served by new antenatal malaria prevention strategies.

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CHANGING DYNAMICS OF MALARIA TRANSMISSION IN RWANDA: AN INTEGRATED EPIDEMIOLOGICAL ASSESSMENT OF DECLINE AND RESURGENCE

Niyonzima Jean Damascene, Habanabakize E. Epaphrodite

Rwanda Biomedical Center, Kigali, Rwanda

Rwanda achieved a substantial reduction in malaria burden over the past decade; however recent data indicate resurgence of Malaria transmission. Understanding the interaction between climatic, behavioral, Parasitological and programmatic factors to inform adaptive control strategies. We conducted a retrospective analysis of national Malaria Surveillance data from FY 2026-2027 to 2024-2025. Indicators included Simple Malaria cases, Annual Parasite Incidence (API), Severe Cases, Malaria related deaths, Slide Positivity Rate (SPR) and Case Fatality Rate (CFR). These trends were triangulated with climatic data, intervention coverage (IRS, ITNs), ITN utilization from MIS and RDHS, Therapeutic Efficacy Study (TES), PfKelch13 mutation prevalence and vector behavior patterns. Malaria burden declined significantly from 2016–2017 to 2022–2023, with cases decreasing from 4.9 million to 0.62 million and API from 418 to 47 per 1,000 population. This decline coincided with expanded vector control and high ITN utilization (69–76%). However, ITN use declined to 58% (children <5) and 61% (pregnant women) in 2019–2020. Evidence of increased outdoor biting behavior suggests reduced effectiveness of indoor interventions. From 2023–2024 to 2024–2025, cases rebounded to >1.06 million (API 76), with increased SPR (14%→21%) and severe cases. The TES showed preserved efficacy of ACTs, but rising Day-3 positivity (up to 15%) and increasing PfK13 mutations (up to 22%) indicate emerging partial resistance. Malaria resurgence in Rwanda reflects a convergence of climatic variability, declining intervention effectiveness, vector behavioral adaptation, reduced ITN utilization, and emergence of partial artemisinin resistance. Rwanda is transitioning toward granular, stratified surveillance (village-level) and targeted, reactive interventions, including focal/reactive IRS, routine and targeted ITNs, RACD in low-burden settings, nationwide passive case management, SBC, and community-led IVM. These adaptive approaches are critical to sustain gains and progress toward malaria elimination.

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TEMPORAL TRENDS OF *PLASMODIUM* SPECIES POSITIVITY RATES ACROSS REGIONS WITH VARYING TRANSMISSION INTENSITIES IN MAINLAND TANZANIA FROM 2021 TO 2023

Happyness A. Mwailunga[1], Baraka W. Mbuba[1], Catherine Bakari[1], Rule Budodo[1], Salehe S. Mandai[1], Laurencia S. Tangawizi[1], Gideoni I. Maulidi[1], Hussein J. Semboja[1], Dativa Pereus[2], Angelina J. Kisambale[1], Ninja S. Bisaga[3], Daniel P. Challe[4], Daniel A. Petro[5], Gervas Chacha[3], Doris Mbata[3], Daniel Mbwambo[6], Sijenunu Aaron[6], Solomon Mwakasungula[1], Abdallah Lusasi[7], Samwel Lazaro[6], Stella Kajange[8], Rashid A. Madebe[3], Celine I. Mandara[3], Seth D. Misago[3], Deus S. Ishengoma[1]

[1]Ifakara Health Institute, Dar es salaam, United Republic of Tanzania, [2]Muhimbili Univesity of Health and Allied Sciences, Dar Es Salaam, United Republic of Tanzania, [3]National Institute for Medical Research, Dar es salaam, United Republic of Tanzania, [4]National Institute for Medical Research, Tanga, United Republic of Tanzania, [5]University of Dar es salaam, Dar es salaam, United Republic of Tanzania, [6]National Malaria Control Programme, Dodoma, United Republic of Tanzania, [7]NMCP, Dar Es Salaam, United Republic of Tanzania, [8]Prime Minister's Office, Regional Administration and Local Government, Dodoma, United Republic of Tanzania

Despite sustained control efforts, routine malaria surveillance in Tanzania predominantly focuses on *Plasmodium falciparum*, with limited evidence on the burden of other species. This study investigated annual trends in *Plasmodium* species across 10 regions of Tanzania, within four transmission strata (very low to high). Health facility cross-sectional surveys were conducted annually (2021 - 2023), samples were collected as dried blood spots (DBS), and DNA was extracted using the Chelex method. Overall, 9119 samples (3221 in 2021, 3552 in 2022, and 2346 in 2023) were analyzed using quantitative PCR (qPCR) targeting the 18s rRNA gene. The overall species-specific positivity rates (PR) declined significantly from 56.8% (n=1,829) in 2021 to 55.3% (n=1,963) in 2022 and 52.3% (n=1,227) in 2023 ($p < 0.001$). *P. falciparum* remained the dominant species but its PR decreased from 55.7% in 2021 to 51.5% in 2023 ($p < 0.001$). *P. ovale* declined significantly from 4.8% in 2021 to 1.5% in 2023 ($p < 0.001$) while *P. malariae* demonstrated a persistent PR, remaining stable at 1.6%, 1.5%, and 1.6% in 2021, 2022, and 2023, respectively ($p = 0.63$), suggesting a stable reservoir that is less responsive to current interventions. *P. vivax* was detected only in three samples in 2021 (0.09%). In the four strata, PR decreased significantly from 70.2% to 59.6%, but remained high in the high-transmission regions (Kagera, Mtwara) ($p < 0.001$). In Moderate regions (Mara, Tabora), PR fell from 76.6% to 61.1% ($p < 0.001$), while in the low (Dar es Salaam, Dodoma, Songwe) and very low strata (Kilimanjaro, Manyara, Njombe) high PR was detected (ranging from 36.8% to 47.6%), suggesting substantial and persistent infections. Adults had the highest proportion of infections (40.1%), but the proportion among under-fives increased significantly from 34.5% in 2021 to 41.2% in 2023 ($p < 0.001$). These findings show that overall PR declined in all regions for both *P.*

falciparum and *P. ovale*, while *P. malariae* remained a stable, hidden reservoir across all transmission strata. Strengthening species-specific molecular surveillance is essential for Tanzania to achieve its 2030 malaria elimination goals.

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COMPLEXITY OF INFECTION AS A MARKER OF CUMULATIVE MALARIA EXPOSURE AND ITS IMPACT ON BURKITT LYMPHOMA RISK

Bing Guo[1], Maia Cheer[2], Namhwa Lee[3], Martin D. Ogwang[4], Patrick Kerchan[5], Steven J. Reynolds[6], Constance N. Tenge[7], Pamela A. Were[8], Robert K. Tenge[7], Walter N. Wekesa[7], Nestory Masalu[9], Esther Kawira[10], Lauryn Butler[1], Tobias Kinyera[11], Isaac Otim[11], Hadijah Nabalende[11], Herry Dhudha[10], Kishor Bhatia[3], James J. Goedert[3], Hyokyoung G. Hong[3], Shannon Takala Harrison[1], Sam M. Mbulaiteye[3]

[1]University of Maryland School of Medicine, Baltimore, MD, United States, [2]University of Maryland School of Public Health, College Park, MD, United States, [3]National Cancer Institute, Bethesda, MD, United States, [4]St. Mary's Hospital, Lacor, Gulu, Uganda, [5]Kuluva Hospital, Arua, Uganda, [6]National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States, [7]Moi University, Eldoret, Kenya, [8]Moi Teaching and Referral Hospital, Eldoret, Kenya, [9]Bugando Medical Center, Mwanza, United Republic of Tanzania, [10]Shirati Health, Education, and Development Foundation, Shirati, United Republic of Tanzania, [11]African Field Epidemiology Network, Gulu, Uganda

Although the risk of childhood Burkitt lymphoma (BL) is highest in regions with intense *Plasmodium falciparum*transmission, the individual-level risk is not well-quantified. Chronic, heavy exposure to diverse *P. falciparum* parasites is hypothesized to increase BL risk, as exposure to new parasite clones results in a germinal B-cell response, somatic hypermutation and rapid B cell proliferation that can lead to BL. Malaria immunity is often non-sterilizing, leading to subclinical, low-parasitemia infections. Since current parasite density may not reflect an individual's prior history, we hypothesized that complexity of infection (COI), the number of unique *P. falciparum* clones, may serve as a more accurate biomarker of the cumulative long-term exposure that drives BL. In this study, we estimated the association between COI and the odds of BL utilizing samples from the Epidemiology of BL in East-African Children and Minors (EMBLEM) case-control study conducted in Uganda, Tanzania, and Kenya, including 500 BL cases frequency-matched by age, sex, and region to 500 community controls. *P. falciparum* positivity was assessed by both microscopy and 18s rRNA qPCR, and COI was estimated using deep amplicon sequencing with a five-marker panel. Among 941 samples, BL cases were significantly less likely to have *P. falciparum* detectable by microscopy (OR=0.46, p<0.01). However, BL cases showed a trend towards having more low-level *P. falciparum* infections as detected only by qPCR (OR=1.21, p=0.29). Among 268 qPCR-positive infections, the effective COI was higher in BL cases (a mean of 10.6 unique clones) compared with controls (a mean COI of 9.0) (OR=1.05, 95% CI 1.00-1.10, p=0.06), adjusting for age, sex, parasite load (qPCR), bednet use, and proximity to surface water. The lower odds of microscopy positivity in BL cases likely reflects greater immunity developed from extensive prior exposure. Our results suggest that determining COI in addition to simple parasite positivity would be a more accurate biomarker for cumulative malaria exposures that drives BL development.

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INE-SCALE SPATIAL EPIDEMIOLOGY OF MALARIA TRANSMISSION HETEROGENEITY IN WESTERN KENYA USING ENSEMBLE MACHINE LEARNING AND HIGH-RESOLUTION REMOTE SENSING DATA

John Uelmen[1], Amy Kristine Bei[2], Eric Ochomo[3], Fang Liu[4], Cristian Koepfli[5]

[1]University of Wisconsin-Madison, Madison, WI, United States, [2]Yale School of Public Health, New Haven, CT, United States, [3]Kenya Medical Research Institute, Kisumu, Kenya, [4]University of Notre Dame, South Bend, IN, United States, [5]University of Notre Dame, Notre Dame, IN, United States

Malaria risk is highly focal, with persistent hotspots identified even within small geographic areas, yet the environmental drivers of this fine-scale heterogeneity remain poorly characterized. We applied ensemble machine learning and high-resolution remote sensing to identify environmental, climatic, and anthropogenic predictors of cluster-level malaria positivity across 20 clusters enrolled in a cluster-randomized spatial repellent trial in western Kenya, where baseline *Plasmodium falciparum* prevalence by qPCR ranged from 26% to 70%. We assembled 459 environmental predictors from multiple satellite platforms, including monthly precipitation (CHIRPS v3.0), daily temperature and wet-bulb globe temperature (CHIRTS-ERA5), land cover and land cover change from ESA Sentinel-2 and GLAD datasets, surface water occurrence and seasonality from the Global Surface Water dataset, and nighttime lights from VIIRS. All variables were extracted within 2-km buffers around cluster centroids, reflecting average daily maximum flight distance for female *Anopheles gambiae*. We modeled cluster-level positivity using bootstrap random forest regression (100 trees, 319 variables sampled per split) and stepwise regression selected by Bayesian information criterion. The random forest explained approximately 69% of variation in positivity rates. Variable importance rankings identified wet-season rainfall, wet-bulb globe temperature, stable tree canopy cover, post-disturbance low vegetation, built-up impervious surfaces, and surface water recurrence as top predictors. Stepwise regression converged on four variables — wetland tree cover, cropland-to-tree transitions, minimum wet-bulb temperature, and maximum precipitation — together explaining 84% of positivity variation. These findings demonstrate that a parsimonious set of remotely sensed environmental predictors can capture substantial spatial heterogeneity in malaria transmission at fine scales, supporting their integration into precision risk mapping and targeted vector control planning in endemic settings.

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MALARIA INFECTIONS AT THE INTERSECTION OF MALNUTRITION, VECTOR CONTROL USE, AND DIVERSE ECOLOGY IN THREE REGIONS OF CAMEROON

Michaela Lynn McGuire[1], Liwang Cui[1], Emma Underwood[2], Chengqi Wang[1], Xiaoyuan Wei[1], Jun Miao[1], Jude Bigoga[3], Christophe Sandra Antonio Nkondjio[4], Akindeh Mbuh Nji[5], Wilfred Fon Mbacham[6], Gillian H. Stresman[1]

[1]University of South Florida, Tampa, FL, United States, [2]University of South Florida, Lutz, FL, United States, [3]Univ of Yaounde I, Yaounde, Cameroon, [4]Manor, PA, United States, [5]University of Yaundé 1, Cameroon, [6]University of Yaounde I, Yaounde, Cameroon

Malaria infection is influenced by environment, vector control use, and individual susceptibility, including malnutrition. While insecticide-treated nets (ITNs) and other vector control methods are vital to malaria prevention, it remains unclear how efficacy differs in malnourished children and the impact improvements in vector control use and malnutrition may have on malaria across distinct ecological zones. Beginning March 2025, quarterly cross-sectional surveys across three regions of Cameroon measured ITN use, malaria via rapid diagnostic test, and mid-upper arm circumference (MUAC) for children five or under. Mixed-effects logistic regression models examined associations between malaria and ITN use, stratified by region. Effect modification by malnutrition status and MUAC were assessed. In addition, scenario-based simulations will be conducted using

standardization and nonparametric bootstrapped uncertainty estimates to project changes in malaria prevalence with hypothetical improvements in ITN use and MUAC. A total of 594 records were included. ITN use, malnutrition, and malaria prevalence varied across regions. Overall, net use was protective for malaria though this was non-significant (OR: 0.71, 95% CI: 0.4-1.26). By region, ITN use reduced malaria prevalence in the Central region (OR: 0.43, 95% CI: 0.21-0.86), no effect was observed in Adamawa region (OR: 0.36, 95% CI: 0.08-1.72), and ITN use increased prevalence in the North region (OR: 6.5, 1.12-37.66). Among malnourished children, ITN use was not protective and may indicate higher odds of malaria, though this was non-significant (OR: 1.48, 95% CI: 0.17-12.84) and data is currently insufficient to evaluate regional differences or perform scenario modeling. Preliminary findings highlight heterogeneous ITN effectiveness for malaria prevention and suggest malnutrition may increase a child's susceptibility. It is therefore plausible tailored interventions to increase ITN use, decrease malnutrition, or a combination may be needed, though continued data collection is needed to refine effect estimates. Final results will be presented at ASTMH 2026.

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MALARIA SURVEILLANCE IN BENIN: FACTORS ASSOCIATED WITH THE DECLINE IN INCIDENCE FROM 2022 TO 2024

Achille Couao-Zotti, Julien Aïssan, Codjo Dandonougbo
National Malaria Control Program (NMCP), Ministry of Health, Benin, Cotonou, Benin

Benin, a West African country of 12.9 million inhabitants, aims to eliminate malaria by 2030. The country has shown a downward trend in malaria incidence between 2022 and 2024. This study analyzes the factors potentially associated with this decline. In this context, we conducted a retrospective analysis of data from the national Malaria Routine Information System (MRIS) covering all 34 health zones. Indicators included annual incidence, confirmed cases, deaths, test positivity rate and biological confirmatio n rate. Trends were examined alongside the deployment of four interventions: monthly routine data validation since 2023, expansion of seasonal malaria chemoprevention (SMC) to 2 additional departments (Borgou and Collines) bringing coverage to all 5 eligible departments (Alibori, Atacora, Borgou, Donga, Collines) and reaching 1,239,864 children aged 3-59 months in 2024, introduction of the RTS,S malaria vaccine in 38 communes (16 health zones) achieving 87.5% coverage for dose 1 and 56.2% for dose 3 by September 2025, and pilot implementation of perennial malaria chemoprevention (PMC). Annual incidence declined from 218 per thousand in 2022 to 174 in 2023 and 139 in 2024, a 36.2% reduction over two years. Confirmed cases decreased from 2,669,335 to 1,739,412 (–34.8%) and deaths from 3,562 to 1,619 (–54.5%). Test positivity rate dropped from 65.5% to 44.1%. Biological confirmation rate reached 99.2% in 2024 versus 97.4% in 2022, while presumptive cases declined by 71.8%. The concurrent decline in both incidence and test positivity rate suggests a genuine reduction in transmission beyond the effect of improved data quality. However, the independent effect of each intervention remains difficult to isolate with this ecological design. A modeling study using the OpenMalaria platform is underway with Swiss TPH to quantify each intervention's specific contribution. These findings support the hypothesis of a combined impact of strengthened surveillance, chemoprevention and vaccination on reducing malaria burden in Benin.

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MALARIA PREVALENCE REACHES HISTORIC LOW ON BIOKO ISLAND IN 2025: EXAMINING PROGRAMMATIC AND ENVIRONMENTAL CONTRIBUTORS

Teresa Ayingono Ondo[1], Federico Comsil Chochi[1], Crisantos Nsue Abeso[1], Charity Okoro[1], Matilde Riloha Rivas[2], Wonder Philip Phiri[1], David Galick[1], Carlos Alberto Guerra[3], Guillermo A. Garcia[4]
[1]MCD Global Health, Malabo, Equatorial Guinea, [2]National Malaria Control Program, Malabo, Equatorial Guinea, [3]MCD Global Health, Quito, Ecuador, [4]MCD Global Health, Hallowell, ME, United States

In 2025, malaria prevalence in children aged 2 to 14 years on Bioko Island reached 7.2%, the lowest ever recorded and the first time prevalence has fallen below 10% since the Bioko Island Malaria Elimination Project (BIMEP) began in 2004. Since program inception, when prevalence stood at 42%, BIMEP has driven a sustained decline through a comprehensive package of interventions. After reaching a low of 12.3% in 2017, prevalence increased in subsequent years, rising from 13.9% in 2023 to 16.9% in 2024 following a delay in island-wide vector control operations that resulted in no indoor residual spraying (IRS) prior to that year's malaria indicator survey (MIS). In response, two IRS rounds were completed between surveys, in October to December 2024 and March to July 2025, alongside LSM expansion into urban Malabo and sustained LLIN distribution across all districts. We will use a household-level linear and multivariable regression analysis to examine the independent contributions of candidate programmatic and environmental factors to the 2025 reduction. Predictors include the number of IRS rounds completed and time elapsed since the last round prior to the survey, insecticide used (broflanilide or clothianidine/deltamethrin), LSM targeting, LLIN access and use, travel frequency, and rainfall derived from NASA remote sensing data. The 2025 reduction represents both a recovery from the 2024 setback and a breakthrough beyond years of stalled progress. Understanding which factors drove this reduction has direct implications for programs elsewhere facing similar transmission plateaus.

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CLIMATE ATTRIBUTED MALARIA TRANSMISSION IN SINDH PAKISTAN, 2018-2024

Amna Tariq
Stanford University, Palo Alto, CA, United States

Pakistan is hyperendemic for malaria and remains the most susceptible country to the repercussions of malaria, with the disease ranking as the fourth-largest cause of death among infectious diseases. In recent years, the profound impact of climate change on malaria has attracted significant attention due to its potential to exacerbate disease burden and alter transmission dynamics. Recently, with increasing temperatures and recurring floodings in Pakistan, subsequent increase in malaria incidence is observed, and it has become imperative to estimate the climate change-attributed increase in the risk of malaria. Data indicates that in 2023, the year after the 2022 flooding event, Sindh observed a 156.2% increase in malaria case incidence compared to 2022. We utilize monthly malaria case incidence data collected by the Sindh Health Department in Pakistan from nine districts from 2018-2024. We are modeling the climate-change and extreme weather (flooding)-attributed malaria transmission in Sindh Pakistan using monthly data on temperature and precipitation. A time-series study design coupled with generalized additive models (GAMs) is utilized to assess the non-linear effects of ambient temperature and rainfall on the risk of malaria transmission in the province. The descriptive analysis highlights mean temperature in Sindh to be 27.8 ˚C (SD: 5.7) and the mean monthly rainfall to be 18.5mm (SD: 50.1) with the maximum rainfall occurring in the months of July and August each year (140-247mm). Preliminary results show that the malaria cases occur at a lag of 4-14 weeks after the rainfall in the province. The GAM explains that the malaria cases rise linearly until temperature reaches 20˚C and then plateaus off. On the other hand, malaria cases decline until rainfall reaches 50mm. After that an increase in malaria cases can be observed until rainfall reaches 150mm and then it plateaus off. Analyses at the district level is currently being processed.

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MEASURING THE IMPACT OF SPATIAL REPELLENTS ON *PLASMODIUM FALCIPARUM* TRANSMISSION POTENTIAL

Delis Mattei-Lopez[1], Colins Oduma[2], Eric Ochomo[3], Cristian Koepfli[1]

[1]University of Notre Dame, Notre Dame, IN, United States, [2]kemri, Kisumu, Kenya, [3]Kenya Medical Research Institute, Kisumu, Kenya

Despite strong malaria control efforts, progress has stalled highlighting the need for novel interventions. In 2025, the WHO promoted the use of spatial repellents to control malaria, the first malaria control recommendation by the organization in 25 years. No research has been conducted to understand the effects these products have on gametocytemia and transmission potential of asymptomatic individuals. We developed, a novel digital PCR assay to quantify transcripts for early-stage (*gexp5*), female (*pfg17*) and male (*pfmget*) gametocytes. We screened samples from repeated cross-sectional surveys conducted within a cluster-randomized trial of spatial repellents in Busia County, Kenya. At baseline, 168/854 (19.7%) individuals carried detectable transcripts for male and female markers. Male gametocytes were detected in highest number of individuals (405/854, 47.4%) while female gametocytes were detected in the lowest number (186/854, 21.8%). The early-stage gametocytes were detected for 356/854 individuals (41.6%). 38/854 of high-density samples (>1000 asexual parasites/µL) did not carry any detectable gametocytes. One year after spatial repellent deployment, even though prevalence of infection did not change, only 93/738 (12.6%) were positive for male and female targets. Comparisons of geometric means of transcripts/µL showed a decrease for each target with a change of -4.3 transcripts/µL for early-stage, -0.52 transcripts/µL for female and -0.6 transcripts/µL for male gametocytes. In both year 1 and year 2, individuals >15 years contributed the most to positive samples, but densities were highest in those <15 years. Differences between intervention and control clusters will be analyzed after unblinding and will be presented.

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RECURRENT AND MIXED *PLASMODIUM* INFECTIONS DURING 12-MONTH FOLLOW-UP OF UGANDAN CHILDREN WITH SEVERE *P. FALCIPARUM* MALARIA

Giselle Lima-Cooper[1], Caitlin A. Bond[2], Daniel Adan Jr[1], Ruth Namazzi[3], Andrea Conroy[4], Robert Opoka[5], Dibyadyuti Datta[1], Chandy C. John[1]

[1]Indiana University School of Medicine, Indianapolis, IN, United States, [2]Indiana University School of Medicine, [3]Makerere University, Kampala, Uganda, [4]Indiana University, Indianapolis, IN, United States, [5]Aga Khan University Medical College, Nairobi, Kenya

Multiple *Plasmodium* species can contribute to malaria morbidity and mortality. In a study of Ugandan children <5 years of age with severe *Plasmodium falciparum* malaria, we documented that 22% of the children had mixed infections *P. ovale* and/or *P. malariae*. However, the prevalence and characteristics of recurrent and multi-species infections among SM survivors following hospital discharge remain poorly characterized. In the present study, we evaluated the prevalence of infection with multiple *Plasmodium* species during visits for clinical malaria during 12-month follow-up in the previously studied cohort of 600 Ugandan children with severe malaria and 120 co-enrolled asymptomatic community children. Children were enrolled and followed from 2014-2018. All children with a history of fever or measured fever during a visit for illness were evaluated for malaria by microscopy and/or RDT. Febrile children positive by microscopy or RDT were further evaluated for *Plasmodium* species infection by nested PCR of filter paper blood spots targeting the 18S ssrRNA gene. All children diagnosed with malaria were treated according to Ugandan Ministry of Health guidelines for malaria. Children with severe malaria did not receive post-discharge malaria chemoprevention, as this was not recommended at the time of study enrollment and follow-up. Among the 556 SM survivors, 274 (49.3%) presented 1 to 15 episodes of recurrent malaria infection during the 12-month follow-up, and 44/274 (16.1%) had mixed infections with a combination of *Pf*, *Po* and/or *Pm*. Among the community children, 42/120 (35.0%) presented with 1 to 6 episodes of recurrent malaria infection during the 12-month period, and 9/42 (21.4%) had mixed infections with a combination of *Pf*, *Po* and/or *Pm*. Our findings emphasize the need for improved detection and treatment of all *Plasmodium* species to reduce malaria burden in children. Evaluation is ongoing to determine if repeat *P. ovale* infections were new or relapses.

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OUTPATIENT COMPLIANCE WITH TEST-AND-TREAT MALARIA GUIDELINES IN NIGERIA: IMPLICATIONS FOR CLINICAL AND LABORATORY QUALITY IMPROVEMENT

Okpe Christian Onuh[1], Justice Baba Adaji[1], Stephen Clinton Uchechukwu[1], Onungbogbo Omowumi[1], Grace Nwabunwanne Nwankwo[1], Aniefiok Akpasa[1], Salamatu Yahaya[1], Zainab Ibrahim[2], Stephen Ake[3], Dan Gadzama Apagu[4]

[1]Famkris Health Care Initiative, Abuja, Nigeria, [2]FCT State Malaria Elimination Program, Abuja, Nigeria, [3]Kogi State Malaria Elimination Program, Lokoja, Nigeria, [4]Health Services and Environment Secretariat, Abuja, Nigeria

Health workers' compliance with clinical guidelines recommending malaria testing of all patients with fever prior to ACT treatment for only test-positive uncomplicated cases (test-and-treat) is the foundation of the outpatient malaria case-management in Nigeria. However, the test-and-treat compliance is not routinely examined, and when occasionally studied, suboptimal practices have been suggested. In this study, prior to the quality improvement intervention, compliance with outpatient test-and-treat standards was cross-sectionally assessed at 29 public facilities in two high malaria risk areas of Nigeria, the Federal Capital Territory (FCT) and Kogi State. The assessments at each health facility were performed by a team of trained assessors using a range of quality-of-care assessment methods. The test-and-treat practices for patients with fever were extracted from the patient cards and facility registers for a one-month period prior to the physical assessments undertaken between October 2025 and February 2026. All health facilities had health workers trained on malaria case-management, all stocked ACTs, 93% had malaria rapid diagnostic tests (RDT), 10% provided malaria microscopy, and 97% had capacity for parasitological diagnosis (RDT or microscopy). Of 2,127 patients with fever, 85.8% were tested for malaria, more commonly by RDTs than microscopy (84.9% vs 15.1%). The test positivity rate was 74.7%, higher for microscopy than RDTs (98.6% vs 70.4%). The treatment compliance for test-positive (ACT) and test-negative patients (no antimalarial) was 94.2% and 93.9%, respectively. The overall compliance, based on composite test-and-treat indicator, was 80.7%. The findings showed relatively high facility readiness and clinical compliance but still existing gaps towards the universal performance targets. The undertesting is the major contributor of the non-compliant test-and-treat practices. The test positivity of malaria microscopy is likely overestimated. The clinical and laboratory quality improvement interventions targeting specific gaps are urgently needed and they will be presented at the conference.

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UNDERSTANDING THE ROLE OF TRANSITORY MIGRATION ON LOCAL MALARIA TRANSMISSION IN INDIGENOUS REGIONS OF THE DARIÉN PROVINCE, PANAMA

Sara M. O'Malley[1], Jose Del Rosario Loaiza[2], Mark M. Janko[3], Gilberto Antonio Eskildsen[4], William Pan[1]

[1]Duke University, Durham, NC, United States, [2]Panama, 8, Panama, [3]Duke Global Health Institute, Carrboro, NC, United States, [4]INDICASAT-AIP, Panama, Panama

Despite recent regional efforts to control and eliminate malaria by 2022, the reduction in malaria transmission has stalled in Central America and, in many cases, is on the rise. In the Darien and Guna Yala regions of Panama, the past 8 years have been marked by an extreme increase in malaria cases from just over 4,000 cases in 2018 to over 15,000 in 2024. Over this same timeframe the region has experienced an extreme rise in

transitory migration from 9,000 individuals in 2018 to over 500,000 in 2023, down to 3,100 in 2025. The majority of new malaria cases in Panama are occurring in indigenous communities, particularly along migratory routes. These same communities also hosted and provided goods and services to migrants as they passed through the Darien region. These events – extreme fluctuation in migration through Panama, the provision of goods, services, and transport in indigenous communities, and increasing malaria rates – are likely interrelated and require further investigation to explain these new dynamics of malaria transmission. This study leverages a case-control study to evaluate the effect of migration on malaria transmission in local communities. Exposure to migration was associated with high odds of malaria infection, particularly in households where two or more individuals sold goods and services to migrants (aOR 14.75, 95% CI 1.67-275.00, p <0.05. Further, individuals who travelled along migration routes in the Darien compared to those that travelled to urban centers had higher malaria risk (OR 4.50, 95% CI 1.12-18.13, p < 0.05). This project supports the central hypothesis that migration and human mobility may contribute to increased malaria transmission in Panama. Ultimately this work provides critical insight into this new malaria landscape in Panama and may help inform malaria control and elimination efforts.

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GENOMIC SIGNATURES OF SELECTION IN *PLAMODIUM FALCIPARUM* FROM SENEGAL

Stephen Schaffner[1], Bassirou Ngom[2], Jonathan Smith[3], Wesley Wong[4], Karina Bellavia[5], Cherif Diouf[2], Djiby Sow[2], Mamadou Alpha Diallo[2], Aita Sene[2], Amy Gaye[2], Mariama Toure[6], Abdoulaye Tine[2], Younouss Diedhiou[2], Jules Gomis[2], Elhadji Doucoure[7], Ibrahima Diallo[8], Standeur Naby Kaly[9], Aliou Thiongane[9], Dyann F. Wirth[10], Bronwyn MacInnis[3], Sarah K. Volkman[10]

[1]Broad Inst of MIT & Harvard, Cambridge, MA, United States, [2]CIGASS, Dakar, Senegal, [3]Broad Institute, Cambridge, MA, United States, [4]T.H. Chan School of Public Health, Boston, MA, United States, [5]Brown University, Providence, RI, United States, [6]CIGASS, DAKAR, Senegal, [7]NMCP, Dakar, Senegal, [8]Senegal Natll Malaria Control Prog, Dakar Fann, Senegal, [9]National Malaria Control Program, Dakar, Senegal, [10]Harvard School of Public Health, Boston, MA, United States

Extensive whole-genome sequence (WGS) data provide a powerful lens to dissect selective forces shaping malaria parasite populations. We analyzed WGS data from 1,503 monogenomic *Plamodium falciparum* infections collected across Senegal (2000-2024) in the context of 10,348 globally distributed monogenomic Pf7 genomes (~900,000 filtered variants) to identify geographically localized signatures of selection. Despite striking differences in transmission intensity between high- (average annual incidence 535‰) and low-transmission (27‰) regions of Senegal, genome-wide allele frequency differences were negligible, with two clear exceptions: the previously reported Pfgvd1 locus on chromosome 9 (CH9) and a locus on chromosome 11 (CH11). At both loci, 1-3 haplotypes were strongly enriched in low-transmission settings, consistent with localized selective pressures favoring these variants, although in neither case has the beneficial phenotype been identified. To test for fitness advantages in low-transmission contexts, we examined associations between haplotypes and the number of clones represented in the dataset (with clonal propagation observed to be much more common at low transmission). Candidate haplotypes at both loci were significantly associated with the presence of clonal partners, with ~4-fold enrichment for CH11 and ~7-fold enrichment for CH9. Haplotypes at CH9 and CH11 were also non-randomly associated with each other and with drug-resistance backgrounds at Pfdhfr, Pfaat1, Pfcrt, and Pfdhps. Geographically, the CH11 haplotype was confined to Senegal, The Gambia, and Mali despite evidence of substantial regional gene flow, whereas the CH9 haplotype was observed in both West and East Africa (including Kenya) and within a clonal cluster in Senegal consistent with recent introduction and expansion of a Kenyan/Tanzanian-like parasite. These findings highlight discrete loci and haplotypes under selection in a low-transmission setting and underscore the value of integrated national and global WGS resources for tracking the emergence and spread of adaptive *P. falciparum* lineages.

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THE GOLDILOCKS EFFECT OF *PLASMEPSIN II*ON RESISTANCE AND FITNESS IN *PLASMODIUM FALCIPARUM* PARASITES

Kelsey N. Murt[1], Biraj Shrestha[1], Abra Rachida Koudjra[1], Lauryn Butler[1], Sudhaunshu Joshi[1], William Witt[1], Emma Rowley[1], Nonlawat Boonyalai[2], Paphavee Lertsethtakarn[3], Piyaporn Sai-Ngam[3], Chaiyaporn Chaisatit[3], Sasikanya Thaloengsok[3], Dysoley Lek[4], Somethy Sok[3], Brian Vesely[3], Mariusz Wojnarski[3], Michele D. Spring[5], Shannon Takala Harrison[1]

[1]University of Maryland School of Medicine, Baltimore, MD, United States, [2]University of Dundee, Dundee, United Kingdom, [3]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [4]The National Center for Parasitology, Entomology and Malaria Control (CNM), Phnom Penh, Cambodia, [5]State University of New York, Upstate Medical University, Dewit, NY, United States

Plasmepsin II (*pmII*) gene copy number is associated with resistance to piperaquine in *Plasmodium falciparum*. However, genome-wide association studies have identified mutations in the *P. falciparum* chloroquine resistance transporter (PfCRT) gene as other drivers of resistance. Gene editing studies have indicated that these mutations are sufficient to confer resistance, but at a fitness cost to the parasite. We hypothesize that *pmII* copy number may modulate the differences in fitness between PfCRT mutations. We obtained Cambodian field isolates (n= 30) harboring PfCRT mutations associated with piperaquine resistance (F145I, G353V, H97Y, and I218F) and grouped them by *pmII* copy number (1, 2 or >2). These isolates were culture adapted and then grown together with an eGFP+Dd2 strain in a growth competition assay using flow cytometry. Three isolates for each genotype were tested when possible. Additionally, piperaquine resistance for these isolates was estimated using the piperaquine survival assay, in which parasites are exposed to the drug for a 48-hour period and parasitemia compared between exposed and non-exposed cultures. We found that PfCRT genotypes differed in fitness in the absence of drug exposure, with H97Y having the highest fitness compared to F145I (the least fit), followed by I218F and G353V. We also found that piperaquine resistance was higher among parasites with 2 or >2 copies of *pmII,* compared to parasites with 1 copy. Additionally, we found that the association between fitness and resistance differed by the number of *pmII* copies, with *pmII* = 2 seeming to confer resistance without a subsequent cost to fitness. This result suggests a goldilocks effect, where 2 copies of the gene is "just right" for the parasite to maintain resistance to piperaquine without sacrificing fitness, consistent with the more rapid decline of parasites with *pmII* >2 and the longer persistence of the *pmII* = 2 after the removal of DHA-PPQ as the first line therapy.

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REGION SPECIFIC *PLAMODIUM FALCIPARUM* JOINT GENOME-WIDE VARIANT CALLING IN SUB-SAHARAN AFRICA

Martha Anita Demba[1], Jonathan Smith[2], Katherine DeRuff[2], Mouhamadou Fadel Diop[1], PAMGEN[3], Bronwyn MacInnis[2], Alfred Amambua Ngwa[1]

[1]MRC Unit The Gambia at London School of Hygiene and Tropical Medicine, Banjul, Gambia, [2]Broad Institute, Cambridge, MA, United States

Accurate detection of genomic variants in *Plamodium falciparum* is critical for understanding the emergence and spread of antimalarial drug resistance and gene flow. Although global reference resources such as MalariaGEN's Pf8 provide extensive variant catalogues, they often underrepresent Sub-Saharan African populations and miss rare, region-specific variations. To address this gap, we performed region-specific joint variant calling on 26200 whole-genome sequences (WGS) using the Terra cloud-based analysis platform. 6402 samples were from twelve Sub-Saharan African countries: The Gambia, Senegal, Mali, Guinea, Sierra Leone, Ghana, Nigeria, Burkina Faso, Ethiopia, Cameroon, Madagascar, and Tanzania. These samples were collected between the years 1966 and 2023 by

PAMGEN and GSM. This dataset includes underrepresented regions alongside 21129 Pf8 samples used as background for comparison. Cloud-based execution enabled scalable processing but required careful optimization to balance computational cost and performance. We adapted the existing Terra variant-calling pipeline to improve efficiency without changing the underlying methods, thereby maintaining accuracy. Cohort-wide joint calling was then performed to produce a comprehensive, unified, high-confidence set of single-nucleotide polymorphisms (SNPs) and small insertions and deletions (indels) in coding and non-coding regions. This approach enabled direct comparison between regional samples and Pf8. We identified variant overlaps and novelty, compared missing genotype rates between datasets, computed allele frequency, and examined population structure, all relative to background variation observed in Pf8. The results highlight region-specific and low-frequency variants that capture local genetic diversity, supporting more accurate population-specific variant analysis and drug resistance surveillance. Overall, we created a scalable dataset that provides a robust, region-specific complement to Pf8, offering actionable insights for malaria control in Sub-Saharan Africa.

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EXPANDING MAD⁴HATTER TO NANOPORE SEQUENCING TO ENABLE DECENTRALIZED MALARIA GENOMIC SURVEILLANCE FOR PUBLIC HEALTH IMPACT IN CHAD

Natasha Turyasingura[1], Aboubacar Ba[2], Xinyan Wei[1], Nicholas Hathaway[3], Olivier Ngaradoum[4,5], Aicha Alhouda Djibrine Oumar[4,5], Mahamat Saleh Issakha Diar[5], Kodbessé Boulotigam[5], Mahamat Idriss Djaskano[5], Perside Lagabka[5], Djiddi Ali Sougoudi[4], Jean-Eudes Djikolmbairangar[5,6], Djamal Hachim Abdoulaye Borgo[7], Abakar Issa Abderamane[7], Mouhamad Saleh[7], Abakar Hassan Gadou[7], Stephane Naboe Jimrane[7], Alex Ndoube Djerabe[7], Houlkeurbe Ngam Daita[7], Mouhamad Abakar Abbass[7], Togou Abate Abakar[7], Aboubakar Hassan Adam[7], Mahamat Mouctar Abdelkerim[7], Moussa Abdoulaye Djibrine[7], Yamti Suitombaye Noubaramadji[8], Koutaya Dezoumbe[8], Meram Yacine Ousmane[9], Nousradine Mahamat Saleh Hassan[10], Abakar Mahamat Tahir[11], Jean Lacsou Teoussou[12], Timothee Marty Bapeng[13], Ahmat Youssouf Ali[14], Ousman Abakar Moussa[15], Tasnim Alhadj Absakine[16], Ali Mahamat Oumar[17], Ramadan Acyl Abderrahim[18], Justine Adjougoulta Koboy Ngo[19], Adirdir Annour[20], Epénétus Wanbane Teugnabé[21], Ali Fadoul Ali[22], Habib Mélé Mahamat[23], Ramadane Arabi Djimet[24], Souleyman Mahamat[25], Prudence Amithara[26], Josaphat Godé[27], Mahamat Zakaria Azrack[28], Meram Atteib Taher[4,9], Adam Batrane[5], Laurent Kodji[29], Kossadoumadji Bendjedid[5], Mahamat Saleh Hamit[5], Mariam Sirbelé[5], Kaltouma Mahamat[5], Mahamat Abakar Allatchéne[30], Alfred Beure Djeguedbe[5], Mahamat Mahamat Saleh Zakaria[5], Abakar Idriss Lawane[5], Abdel-Razik Ibrahim[4], Hissein Mougouni Mahamat[5], Mahamat Moussa Hassan Taïsso[4,5], Mahamat Adano Adoudou[5], Hassoumi Manah[5], Issa Ali Haggar[5], Brahim Adoum[5], Idriss Mahamadou Zezerti[5], Adjabo Djibrine[5], Mahamat Issa Ratou[5], Issa Ramad[5], Jessica Briggs[3], Bryan Greenhouse[3], Margaret Anderson[1], Kaylee S. Herzog[31], Vidushi Kapoor[32], David Debruyne[32], Niyati Thosani[32], Joseph Fauver[31], Andrés Aranda-Diaz[3,33], Mahamat Fayiz Abakar[9], Sten H. Vermund[34], Hassan Mahamat Ali[7,9], Ali Mahamat Moussa[9], Issa Mahamat Souleymane[4,5], Amy K. Bei[1,2]

[1]Yale School of Public Health, New Haven, CT, United States, [2]Institut Pasteur de Dakar, Dakar, Senegal, [3]University of California, San Francisco, CA, United States, [4]University of N'Djamena, N'Djamena, Chad, [5]National Malaria Control Program, Ministry of Public Health, N'Djamena, Chad, [6]Vector Borne Infectious Diseases Laboratory of the Research Unit for Biology and Applied Ecology, Department of Animal Biology, Faculty of Science, University of Dschang, Dschang, Cameroon, [7]Laboratory for Biosecurity and Epidemics, N'Djamena, Chad, [8]Laboratory for Major Tropical Epidemics, N'Djamena, Chad, [9]Institut National de Santé Publique du Tchad, N'Djamena, Chad, [10]Centre Hospitalier Universitaire de la Mère et de l'Enfant, N'Djamena, Chad, [11]National Malaria Control Program, Mayo Kebbi Est, Chad, [12]National Malaria Control Program, Mayo Kebbi Ouest, Chad, [13]National Malaria Control Program, Logone Occidental, Chad, [14]National Malaria Control Program, Faya, Chad, [15]National Malaria Control Program, Bol, Chad, [16]National Malaria Control Program, Am-Timan, Chad, [17]National Malaria Control Program, Moussoro, Chad, [18]National Malaria Control Program, Mao, Chad, [19]National Malaria Control Program, DS Sud, Chad, [20]National Malaria Control Program, Kelo, Chad, [21]National Malaria Control Program, Koumra, Chad, [22]National Malaria Control Program, Massaguet, Chad, [23]National Malaria Control Program, Dourbali, Chad, [24]National Malaria Control Program, Mongo, Chad, [25]National Malaria Control Program, Abeché, Chad, [26]National Malaria Control Program, Adré, Chad, [27]National Malaria Control Program, Goz Beda, Chad, [28]Toumaï University, N'Djamena, Chad, [29]Centre de Contrôle de la Qualité des Denrées Alimentaires, N'Djamena, Chad, [30]National Malaria Control Program, Ndjamena Bilala, Chad, [31]University of Nebraska Medical Center, Omaha, NE, United States, [32]Paragon Genomics, Inc., Fremont, CA, United States, [33]ISGlobal, Barcelona, Spain, [34]University of South Florida, Tampa, Florida, United States

Despite multiple control interventions by Chad's National Malaria Control Program (PNLP), malaria remains a public health challenge in Chad. A PNLP priority is therefore to evaluate the efficacy of deployed interventions and explore genomic tools to characterize circulating malaria parasite genotypes within Chad to inform public health policy. Country-level data on parasite diversity and resistance are critically lacking. In partnership with the PNLP, we employed the Multiplexed Amplicons for Drugs, Diagnostics, Diversity and Differentiation via High Throughput Targeted Resequencing (MAD⁴HatTeR) pipeline: a modular tool designed to run on the Illumina platform but adapted here to be compatible with library preparation and sequencing by Oxford Nanopore Technology (ONT). 1,160 RDT-positive clinical isolates of unknown parasitemia were collected from 20 provinces in 2024-2025. DNA was extracted from dried blood spots, PCR amplified with MAD⁴HatTeR pool R1.2 primers adapted for ONT and barcoded by PCR ligation. Libraries were sequenced on the portable MinION sequencer. Super high accuracy basecalled fastq files were analyzed using the SeekDeep pipeline for haplotype frequency estimation. Of 192 samples processed, read counts per sample varied across provinces; most samples achieving 70% passing reads. While *pfcrt* and *pffd* genes remained challenging targets, the MinION recapitulated previous findings with the chloroquine resistance IET haplotype observed in 52% of samples, N51I, C59R and S108N *pfdhfr* mutations present at >90% allele frequency and the combined quintuple mutation haplotype identified in 10% of samples, highlighting compromised sulfadoxine-pyrimethamine treatment efficacy. While most *pfKelch13* alleles were wild-type, the validated A675V mutation was detected in a single sample with high confidence. 9 samples were identified with *pfhrp3* deletions and 2 isolates revealed non-*falciparum* species (both *Plasmodium ovale curtisi*). This pilot study demonstrates the feasibility of the MAD⁴HatTeR panel on ONT technology, offering a pathway for decentralized, real-time malaria genomic surveillance in Chad.

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COMPUTATIONAL PHASING OF POLYCLONAL MALARIA INFECTIONS FROM TARGETED AMPLICON SEQUENCING DATA

Maxwell Murphy, Nicholas Hathaway, Bryan Greenhouse

Univ of California San Francisco, San Francisco, CA, United States

Resolving haploid *Plasmodium* haplotypes within polyclonal malaria infections can greatly facilitate tracking drug resistance, identifying imported cases, and characterizing transmission. However, existing phasing methods fail for malaria due to unknown complexity of infection (COI) and reliance on whole genome sequencing (which lacks sensitivity for minor clones) or biallelic single nucleotide polymorphisms (which ignores linkage information). Targeted amplicon sequencing (TAS) generates quantitative allele proportions at highly diverse microhaplotype loci and is widely deployed in malaria genomic surveillance, yet no computational methods exist to leverage these data for within-infection haplotype phasing. We developed a computational framework to phase haplotypes from TAS data that jointly infers the number of distinct clones, their full haplotype sequences across all genotyped loci, and their relative abundances within each infection. Our approach uses an expectation-maximization algorithm with stochastic search to maximize a multinomial likelihood over observed read counts at each locus. In simulations across a range of COI (25 loci, 10 alleles/

locus, 10,000 reads/locus, 20 replicates per COI level), median haplotype assignment accuracy was ≥0.92 for COI 2-5 (1.0 for COI 2-3), with graceful degradation at higher complexity (median 0.80 at COI 6, 0.43 at COI 10). Mixture proportion estimation remained accurate across this range, with median absolute error below 0.02 for COI 2-5 and below 0.05 through COI 7. We validated our method on 24 laboratory control mixtures of known strain composition (COI 3-7) genotyped at 166 microhaplotype loci. After filtering globally inconsistent loci, median assignment accuracy was 0.90 at COI 3, 0.71 at COI 5, and 0.65 at COI 7. Performance was also influenced by parasite density, with lower-density samples showing reduced phasing accuracy. Our framework will be released as an open-source R package, enabling haplotype-level analysis from widely available TAS data to support drug resistance surveillance and malaria transmission characterization.

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GENOMIC ANALYSIS OF *PLASMODIUM FALCIPARUM* INVASION LIGANDS AND HUMAN RECEPTORS IDENTIFIES SIGNATURES OF MALARIA SUSCEPTIBILITY IN THE GAMBIA: INSIGHTS FROM NANOPORE SEQUENCING

Nora Nghochuzie Nganyewo[1], Marther Anita Demba[1], Fatoumatta Bojang[1], Mitchel Katsvanga[1], Lucas Amenga-Etego[2], Alfred Amambua-Ngwa[1]

[1]MRCG@LSHTM, Banjul, Gambia, [2]WACCBIP, Accra, Ghana

The malaria parasite *Plasmodium falciparum* utilizes multiple pathways to invade the red blood cells (RBCs) and this stage of the parasite's life cycle is responsible for the clinical signs and symptoms of the disease. Interactions between the human receptors and parasite ligands during this process might have resulted to mutations in genes involved in these pathways which can affect the outcome of the disease. Thus, dual genetic studies looking at evolutionary changes in genes involve in both the host receptors and parasites ligands during erythrocyte invasion are useful in understanding the parasite's biology and development of drugs and vaccines. This study made use the Oxford Nanopore Technology (ONT) approach to sequence a panel of 16 genes using DNA samples from 188 malaria positive (165 mild and 23 severe) individuals from four sites (Brikama, Basse, Fajikunda, and EFSTH) in The Gambia. This panel included 12 parasite genes (Clag 2 and 8, EBA140, 175 and 181, Rh4, Rh5, MSP1 and 6, EBL1, SURFIN4.2, DBLMSP), and 4 human receptors (complement receptor1 (CR1), glycophorin (GP) A, B and C). After quality control, the data was mapped to the human (GRCh38) and *P. falciparum* (PlasmoDB v.9.0 3D7) reference genomes and the mapped reads were used for downstream analysis. Population structure analysis for the receptors showed subtle structure with individuals from Brikama clustering together while no population structure was observed between parasites from the four sites. Furthermore, SURFIN4.2, and DBLMSP showed high inter-SNP linkage disequilibrium (LD) for the parasite population while the three human receptors (CR1, GPB and GPC) also indicated high levels of LD. Additionally, a matched case-control analysis between severe and mild malaria identified a single SNP in SURFIN4.2 and few SNPs in CR1 gene associated with malaria susceptibility indicating that variations in both the human host and parasite ligands may determine the outcome of the disease and dual genetics studies are important in malaria control and elimination.

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GENETIC SURVEILLANCE MIRRORS SUB-NATIONAL MALARIA BURDEN IN SENEGAL GENETIC SURVEILLANCE MIRRORS SUB-NATIONAL MALARIA BURDEN IN SENEGAL

Djiby Sow[1], Bassirou Ngom[1], Wesley Wong[2], Mamadou Alpha Diallo[1], Amy Gaye[1], Aita Sene[1], Abdoulaye Tine[1], Mariama A. Toure[1], Cherif Diouf[1], River Mallick[3], Younousse Diedhiou[1], Jules Gomis[1], Mouhamadou Ndiaye[1], Mame Cheikh Seck[1], Khadim Diongue[1], Aida Sadikh Badiane[1], Daba Zoumarou[1], Mamadou Samb Yade[1], Elhadji A. Doucoure[4], Baba Dieye[1], Ibrahima Diallo[4], Medoune Ndiop[4], Standeur Naby Kaly[4], Aliou Thiongane[4], Bronwyn Macinnis[5], Dyann F. Wirth[3], Daouda Ndiaye[1], Sarah K. Volkman[3]

[1]Centre International de Recherche et de Formation en Génomique Appliquée et de la Surveillance Sanitaire, Dakar, Senegal, [2]T.H. Chan School of Public Health, Boston, MA, United States, [3]Harvard T.H. Chan School of Public Health, Broad Institute, Boston, MA, United States, [4]National Malaria Control Program (NMCP), Senegal, Dakar, Senegal, [5]Broad Institute, Cambridge, MA, United States

Timely, reliable subnational malaria surveillance is essential for tracking transmission and targeting interventions, yet routine incidence data in Senegal are often incomplete, delayed, or difficult to compare across sites and years. We evaluated whether parasite genetic data can serve as a scalable, independent proxy for transmission intensity. Using 3,147 *Plasmodium falciparum* clinical isolates collected from 16 health facilities between 2012 and 2020, we trained a Poisson generalized linear mixed-effects (GLM) model to predict annual incidence (cases per 1,000 population) from five genetic metrics derived from a 24SNP barcode. The model showed strong agreement with National Malaria Control Program (NMCP) incidence (AIC = 1291.34). We then extended this framework to amplicon sequencing (AmpSeq) data using a jointcalibration strategy that retrained the model on both barcode (2012–2020) and AmpSeq (2020) datasets, yielding a strong correlation (R^2 = 0.53) between genetic-predicted and NMCP reported incidence for independent AmpSeq data. Here we expand testing this predictive model and applied the retrained GLM model to independent AmpSeq data from 900 infections at 12 health posts (2023) and 1,519 infections from 14 health posts (2024) to show geneticpredicted incidence remained strongly correlated with NMCP incidence (Pearson r = 0.64, p < 0.001; $\log_{10}$transformed r = 0.38, p = 0.027). Most sites showed close concordance between genetic and NMCP estimates, while a few discordant locations (Bandafassi, Nianao) flagged possible focal transmission changes, importation, or reporting gaps. To further assess the utility of AmpSeqbased genotyping, we will use residualbased models to test whether haplotype diversity metrics (e.g., Moiré, h2/h1) improve genetic incidence prediction compared with our previous model, which relied solely on SNPbased metrics. These findings show that parasite genetic data can robustly capture spatial and temporal variation in malaria burden and provide an operational, samplebased surveillance tool to guide sub-national control and elimination efforts in Senegal.

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DEVELOPMENT OF NOVEL GENETICALLY DIVERSE *PLASMODIUM FALCIPARUM* STRAIN TO USE IN CONTROLLED HUMAN MALARIA INFECTION (CHMI) STUDIES

Elgin H. Akin[1], Kathleen Moch[1], Medhini Ramesh[1], Charvita Nemarugommula[1], Mariah L. Desroches[1], Ben M. Andagalu[2], Hoseah M. Akala[2], Edwin Kamau[1], **Alexander V. Pichugin**[1]

[1]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [2]Kenya Medical Research Institute/Walter Reed Army Institute of Research - Africa, Kisumu, Kenya

Genetic diversity in *Plasmodium falciparum* (*Pf*) antigens poses significant challenges for malaria vaccine design and efficacy testing in Controlled Human Malaria Infection (CHMI) studies. Current CHMI strains capture limited global diversity, restricting the evaluation of anti-malaria vaccines. To address this, we analyzed clinical isolates from Ghana, Kenya, Cambodia, and Colombia. Samples were genotyped at 12 microsatellite loci and

underwent targeted sequencing of circumsporozoite protein (CSP) and apical membrane antigen 1 (AMA1). Our genetic analysis revealed strong geographic structure and high allele richness, particularly in African isolates. CSP exhibited extensive N-terminal structural variation, diverse central repeat region (CRR) compositions, and polymorphism in the C-terminal domain. AMA1 diversity was highest in the hypervariable domain but extended across domains I and II. Importantly, CSP and AMA1 diversity is poorly represented by current CHMI strains, risking biased vaccine efficacy estimates. To address this gap, we culture-adapted and cloned Kenyan isolates, testing them for gametocyte and sporozoite production, drug sensitivity, and infection of primary human hepatocytes. We downselected and completed cGMP bio-production of a novel heterologous *Pf* strain of Kenyan origin, K12-6, for use in CHMI studies. Based on our analysis of the CSP CRR, the K12-6 haplotype is the most common, representing 61% of our total samples and 91% of African samples. In contrast, the 3D7/NF54-like haplotype is much rarer, found in only 4% of total samples and 4.7% of African samples. Compared with the NF54/3D7-derived CHMI strains currently in use, K12-6 displays distinct CSP and AMA1 haplotypes representative of common African parasite lineages, thereby expanding the antigenic diversity evaluated in CHMI-based vaccine trials. The inclusion of this strain in the current CHMI panel addresses a critical gap identified by the FDA, which has been a significant barrier to leveraging CHMI studies for the licensure of anti-malarial products that protect travelers and warfighters deployed to malaria-endemic regions.

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ANEUPLOID MALARIA PARASITES: FREQUENCY, STABILITY, FITNESS AND TRANSCRIPTIONAL IMPACT

Shalini Nair[1], Michael H. Nipper[1], Xue Li[1], Grace Arya[1], Francis Nkemngo[1], Sudhir Kumar[2], Lisa A. Checkley[3], Biley A. Abatiyow[4], Stefan Kappe[4], Ashley Vaughan[4], Michael Ferdig[3], Ian H. Cheeseman[1], **Timothy JC Anderson**[1]

[1]Texas Biomedical Research Institute, San Antonio, TX, United States, [2]Iowa State University, Ames, IA, United States, [3]University of Notre Dame, South Bend, IN, United States, [4]Seattle Children's Research Institute, Seattle, WA, United States

Aneuploidy is frequent in parasitic protozoa such as Leishmania where it plays an important role in adaptation. However, aneuploidy has not been reported in malaria parasites to our knowledge. Here we describe aneuploid parasites identified from sequencing 1497 F1 progeny parasites from three genetic crosses. We identified 3 (0.2%) aneuploid parasites from analysis of sequence read depth. These carried two copies of either chromosome 1 or 3. In two cases, aneuploid parasites were found among self-fertilized progeny, while the third was in an outcrossed progeny. In all cases we found identical-by-descent (IBD) parasites lacking aneuploidy providing controls. We cultured aneuploid parasites for 1-2 months to evaluate stability. In all three cases aneuploidy was unstable and was strongly selected against. We are currently examining single cell transcriptomic data from aneuploid and matched IBD control parasites to examine how transcription is impacted on aneuploid chromosomes and elsewhere across the genome. We suspect that aneuploidy may occur frequently during malaria parasite cell division, but that aneuploid parasites are rarely recovered because chromosomal duplications are unstable and deleterious.

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GENETIC DIVERSITY OF *PLASMODIUM FALCIPARUM* IN *ANOPHELES STEPHENSI* INVADED REGIONS OF EASTERN ETHIOPIA

Edom Seyoum[1], Elizabeth Waymire[1], Isuru Gunarathna[1], Edilawit Tiruneh[2], Solomon Abay[3], Solomon Yared[4], Tamar E. Carter[1]

[1]Baylor University, Waco, TX, United States, [2]Jijiga University, Jigjiga, Ethiopia, [3]Addis Ababa University, Addis Ababa, Ethiopia, [4]Jigjiga, Ethiopia

Malaria transmission depends on the successful invasion of *Plasmodium* parasites into the mosquito midgut, a process governed by vector-parasite compatibility and mediated by parasite surface interaction genes. Majority of malaria infections in Africa are caused by *Plasmodium falciparum,* which is responsible for most severe cases and mortality. Surface interaction genes including *Pfs47, Pfs16* and *Pfs25* play a key role in evading mosquito immune response and enabling the parasite development within the vector. The recent invasion of *Anopheles stephensi* into eastern Ethiopia represents a major shift in regional transmission dynamics and imposes selective pressure on circulating parasite populations. Although these interaction genes have been well characterized in mosquito stages, the genetic diversity in circulating human infections remains unknown. To address this, we analyzed *P. falciparum* isolates from 60 dried blood spot (FTA card) samples collected in Erer, Hurso and Bike, Ethiopia which are regions with an established *An. stephensi* presence. Targeted sequencing of *Pfs47*, *Pfs16,* and *Pfs25* were performed to assess haplotype diversity, parasite population structure, and geographic distribution. Preliminary findings of *Pfs47* identified 11 distinct haplotypes among our samples, demonstrating measurable genetic diversity. Further analysis will evaluate whether haplotype distributions are non-random and exhibit geographic clustering consistent with vector-mediated selection. These findings will provide insight into how vector invasion reshapes parasite population structure and transmission dynamics.

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ROBUST, REPRODUCIBLE BENCHMARKING OF BIOINFORMATIC TOOLS FOR MALARIA GENOMIC EPIDEMIOLOGY

Nicholas Hathaway[1], **Alfred B. Hubbard**[2], Kathryn Murie[1], Sophie Bérubé[3], Zoe Gotthold[4], Bing Guo[5], Jason A. Hendry[6], Maxwell Murphy[1], Bassirou Ngom[7], Josphat N. Nyataya[8], Shazia Ruybal-Pesántez[9], Aimee R. Taylor[10], Robert Verity[11], Bryan Greenhouse[1], Amy Wesolowski[12]

[1]University of California, San Francisco, San Francisco, CA, United States, [2]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [3]University of Florida, Gainesville, FL, United States, [4]National Institute of Allergy and Infectious Disease, Bethesda, MD, United States, [5]University of Maryland School of Medicine, Baltimore, MD, United States, [6]Max Planck Institute for Infection Biology, Berlin, Germany, [7]CIGASS, Dakar, Senegal, [8]Kenya Medical Research Institute (KEMRI)/Walter Reed Army Institute of Research-Africa, Kisumu, Kenya, [9]Barcelona Institute for Global Health, Barcelona, Spain, [10]Institut Pasteur, Paris, France, [11]Imperial College London, London, United Kingdom, [12]Johns Hopkins University, Baltimore, MD, United States

Numerous computational tools have been developed to estimate epidemiologically important quantities from malaria genomic data, such as complexity of infection (COI), population allele frequencies, and between-sample relatedness. However, systematic and standardized benchmarking of these tools has never been performed, leaving researchers without clear guidance on which tools perform optimally across datasets and epidemiological settings. The absence of standardized evaluation also means that no framework exists to benchmark future tools against existing options, making it challenging to properly evaluate advances. To address these gaps, we developed a reproducible, open-source benchmarking pipeline for malaria genomic epidemiology tools. The pipeline includes simulation of malaria populations with the *recombuddy* R package, simulation of PCR and genotyping, amplicon calling, and evaluation of each statistical tool of interest on this simulated data. We applied this pipeline to the current landscape of tools that estimate COI and/or population allele frequencies, including a suite of naive, model-free methods that we developed. The results of this initial benchmarking revealed that, in some contexts, naive approaches perform as well as more complex statistical models. Our results provide guidelines for selecting the appropriate tool based on the metrics of interest, data properties, and epidemiological setting. These results are publicly available online as part of the PGEforge website, as are instructions for replicating this analysis and links to the various components of the pipeline. With this framework, benchmarking can easily be accomplished for new tools or applications and the results promptly uploaded to the PGEforge website. This work establishes best analytic practices based on quantitative comparisons and supports the growth of a robust and sustainable tool ecosystem.

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WHOLE-GENOME SCANS FOR COPY NUMBER VARIATION IN GLOBAL *PLAMODIUM FALCIPARUM* POPULATIONS

Jacob W. Marglous, Jeffrey A. Bailey
Brown University, Providence, RI, United States

In recent years, *Plasmodium falciparum* resistance mutations to artemisinin and its partner drugs in artemisinin combination therapies (ACTs) have emerged and are spreading rapidly in Africa, where the vast majority of morbidity and mortality occur. While most analyses of parasite genetics focus on single nucleotide polymorphisms (SNPs), copy number variants (CNVs) are significant contributors to the parasite's overall genomic variation and are known mediators of drug resistance. For example, duplications in Southeast Asia of *mdr1* and *plasmepsin2/3* are associated with mefloquine and piperaquine resistance, respectively. However, CNVs remain understudied, particularly in African populations, in part because their detection is challenging using short read sequencing. Here, we further adapt a hidden Markov model-based method for CNV detection in whole-genome and whole-exome sequencing, designed for humans as a component of the Genome Analysis ToolKit (GATK). We refine this method, previously used to analyze only known drug resistance loci in *Pf*, for application across the genome to over 20,000 well-sequenced samples in the recently released Pf8 dataset. In a notable improvement over previous applications, we call integer copy number states as well as variant breakpoints. We incorporate PCA clustering prior to genotyping to improve model performance. Filtering thresholds were optimized to maximize sensitivity and specificity on samples with known CNV. We confirm previous reports of low overall prevalence of CNV in key drug resistance genes in African parasite populations. We also scan for novel CNV occurring at increasing frequencies in specific geographic regions. Future directions include assessment of novel CNV loci for additional signatures of selection and in vitro validation of these potential drug resistance markers. These results motivate further development and application of methods to detect CNV in emerging African genomic surveillance data, towards rapid detection of partner drug resistance and mitigation of the threat it poses to ACT efficacy.

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MODELLING WITHIN HOST SELECTION DYNAMICS AND COMPETITIVE RELEASE IN *PLASMODIUM CHABAUDI*: IMPLICATIONS FOR OPTIMAL TREATMENT STRATEGIES

Chemia Moayyed, John Kornak, Bryan Greenhouse, Suzanne M. Dufault
University of California, San Francisco, San Francisco, CA, United States

The rise of multidrug-resistant *Plasmodium* strains threaten to cause an additional 16 million cases and 80,000 deaths annually according to the WHO. Treatment strategies have predominantly favored more aggressive regimens, partly to minimize de novo mutations, but these strategies have shown to induce rapid selection for pre-existing resistant strains. While previous analyses have focused on population scale resistance, in this work we analyze within host dynamics of selection toward resistant mutants and relapse trajectories by performing a secondary analysis of a dataset from a 2013 study involving P. *Chabaudi* infected mice. The original study utilized 32 mice inoculated with a mixture of 10^6 drug-susceptible and 10 drug-resistant genotypes to simulate early-stage resistance emergence, and grouped by antibiotic (Pyrimethamine) intensity into 3 groups in addition to an untreated group. Measurements of genotype densities as well as red blood cell (RBC) count were monitored longitudinally for 47 days. We used the data from the erythrocytic cycle and built on the Lotka-Volterra competition model, modifying it to align with the specifications and assumptions we hold for the current system. We define rate of change of the genotypes in each other's presence, stratifying on states of drug presence relative to its effectiveness threshold, creating a binary condition for each observation. Using systems of differential equations and defining competition modalities, we were able to narrow down the equilibrium profile structure guided by results of data fitting. Our regression results on rate of change revealed paradoxical effects of drug presence, indicating evidence for involvement of an additional entity playing a role in the structure and trajectories of the system. We hypothesize the additional entity to represent adaptive immune processes, with a multiphase and asymmetric involvement towards the different genotypes based on density dominance of mutants, and we mapped the interplay between antibiotic introduction and the existing immune guard. Our findings further emphasizes the necessity of revisiting optimal strategies.

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(UNIQUE MOLECULAR IDENTIFIERS) UMI-CAPSEQ: ACCURATE HAPLOTYPE RESOLUTION OF MULTICLONAL *PLASMODIUM* INFECTIONS USING UMI-BASED CAPTURE SEQUENCING

Elizabeth Hemming-Schroeder
Colorado State University, Fort Collins, CO, United States

One barrier to accurately reconstructing Plasmodium transmission chains is assigning haplotypes in multiclonal infections. Current sequencing approaches rarely enable direct haplotype reconstruction in complex infections, introducing additional uncertainty in downstream kinship inference. Most methods rely on likelihood-based estimates of genetic distance between polyclonal infections (e.g., Dcifer), rather than direct observation of parasite haplotypes. Typically, parasite enrichment approaches are necessary to perform due to the high amount of contaminating host DNA, but these often introduce amplification bias as strains in a multiclonal infection amplify at different rates across loci. Thus, even if parasite clones are abundant in a sample at distinct proportions, the proportion of sequencing reads at each locus does not correspond to the relative parasite abundance in the sample. Here, we present a novel library preparation and sequencing approach designed to retain information about the relative parasite abundance across loci. This approach, termed UMI-CapSeq involves ligating unique molecular identifiers (UMIs) prior to parasite enrichment, enabling molecule-level tracking through downstream processing. Following genomic fragmentation, DNA fragments are ligated to UMI-UDI adapters, enriched using biotinylated capture baits targeting parasite loci, and sequenced using long-read technology. By tagging molecules prior to enrichment, UMI-CapSeq mitigates issues of amplification bias by preserving original parasite strain abundance in the number of unique UMIs per locus and strain. Preliminary data indicate improved concordance between molecular counts and underlying strain abundance as well as supports the feasibility of this approach for genotyping parasites from dried blood spots. Ongoing analyses are quantifying method performance, including accuracy and consistency across loci. This framework has the potential to improve haplotype resolution and strengthen kinship inference in multiclonal infections, ultimately enhancing our ability to reconstruct Plasmodium transmission networks.

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PLASMODIUM VIVAX GAMETOCYTE DYNAMICS IN SYMPTOMATIC AND ASYMPTOMATIC INFECTIONS

Tassew Tefera Shenkutie[1], Beka R. Abagero[1], Chiekh Cambel Dieng[1], Ikechukwu Chidiebere Nwankwo[1], Meshesha Tsigie Negash[2], Lea Baldor[3], Brice Feufack Donfack[3], Hangjun Ke[4], Abnet Abebe[2], Jean Popovici[5], Eugenia Lo[1]
[1]Drexel University, Philadelphia, PA, United States, [2]Ethiopian Public Health Institute, Addis Ababa, Ethiopia, [3]Institut Pasteur du Cambodge, Phnom Penh, Cambodia, [4]Drexel Univ Coll of Medicine, Philadelphia, PA, United States, [5]Institut Pasteur of Cambodia, Phnom Penh, Cambodia

Gametocytogenesis in *Plasmodium vivax* (*Pv*) is characterized by early development, continuous production, and high infectivity to mosquito vectors. Sexual commitment is regulated by transcription factors and epigenetic mechanisms, in which *Gametocyte Development 1* (*GDV1*) activates *AP2-G*, the master regulator of sexual conversion. Parasite genetic diversity and multiplicity of infection (MOI) are among the factors

that may influence gametocyte production. While transmission from submicroscopic infections has been reported, the expression dynamics of gametocyte development genes and associated factors remain poorly characterized in symptomatic versus asymptomatic *Pv* infections. This study compared gametocyte carriage, parasite haplotype diversity, and the expression of gametocyte development genes. Total RNA was extracted from TRIzol-preserved blood samples, followed by cDNA synthesis and quantitative RT-PCR. A total of 221 PCR-confirmed *Pv* infections were analyzed, including 101 symptomatic and 120 asymptomatic cases. Gametocyte prevalence was higher in symptomatic individuals (100%) than in asymptomatic individuals (59.7%); however, no significant difference was observed in the relative expression of *Pvs25* between the two groups. Similarly, the relative expression of the sexual commitment genes, *AP2-G* and *GDV1* did not differ, suggesting comparable rates of gametocyte conversion. *PvMSP1*-based amplicon deep sequencing revealed that Increase in parasite clones within an infection did not increase gametocyte abundance. However, individual clones observed in monoclonal infections varied by gametocyte production and sexual commitment, implicating differences in transmissibility. Overall, detectable gametocyte density and sexual commitment in asymptomatic infections highlight their potential contribution to malaria transmission. The lack of correlation between sexual commitment markers and mature gametocyte density suggests a possible regulatory gap during gametocyte maturation that warrants further investigation.

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POTENTIAL RISK OF MALARIA TRANSMISSION ON ANTIMALARIAL TREATMENT WITH EVIDENCE OF *PLASMODIUM VIVAX* DEVELOPMENT IN *ANOPHELES STEPHENSI*, A CHALLENGE FOR MALARIA ELIMINATION

Alex Eapen, Sangamithra Ravishankaran, Aswin Asokan
ICMR-National Institute of Malaria Research, Chennai, India

The global malaria burden continued to rise in 2024, with an estimated 282 million cases across 80 endemic countries, marking an increase of about 9 million cases (3%) compared to the previous year. Factors such as global warming and climate change disruptions, shifts in rainfall patterns, emerging insecticide-resistant mosquitoes, and drug-resistant parasites influence the life history traits of both the mosquito and the parasite. This complicates the understanding of the vector-host-parasite relationship and hinders efforts to eliminate the disease. The current study aimed to determine whether patients undergoing antimalarial treatment remain at potential risk of transmitting the infection if they are unprotected from vector bites. Blood infected with *Plasmodium vivax*, collected from malaria patients before treatment and after 24 hours of antimalarial therapy, was membrane-fed to *Anopheles stephensi mosquitoes*. The development of malaria parasites before and during treatment was monitored in *An. stephensi*, simulating the microclimatic conditions of the prevailing household roof types in incubators, along with standard laboratory conditions. The development of oocysts and sporozoites was observed under the temperature conditions characteristic of simulated roof types for blood infected with *P. vivax* before treatment. Thatched-roof conditions proved most favourable for parasite development, with higher oocyst counts and longer sporozoite persistence. Surprisingly, parasite development to the infective stage, sporozoites, was detected in membrane-fed blood after 24 hours of treatment with antimalarial drugs in *An. stephensi*. The study highlighted a potential risk of malaria transmission even on antimalarial therapy in hypo-endemic areas if patients are not protected, do not use insecticide-treated nets, and are exposed to early and outdoor vector bites, posing a significant challenge for malaria elimination efforts.

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PREVALENCE OF *PLASMODIUM FALCIPARUM* AND HELMINTHS INFECTION AMONG SCHOOL CHILDREN IN TWO SELECTED COMMUNITIES IN KASSENA NANKANA MUNICIPALITY

Millicent Captain-Esoah[1], Williams Ayaaba[1], Emmanuella Captain Esoah[2], Kwadwo Kyereme Frempong[3], Martin Ntiamoah Donkor[4], Grace A. Motey[1], Mawuli Kwamla Azameti[1], Sylvester Donne Darsah[4]
[1]Deptartment of Applied Biology, School of Environment and Life Sciences, University of Technology and Applied Sciences, Navrongo, Ghana, [2]Department of General and Preventive Health Nursing, School of Nursing and Midwifery, University of Technology and Applied Sciences, Navrongo, Ghana, [3]Department of Parasitology, Noguchi Memorial Institute for Medical Research (NMIMR), College of Health Sciences, University of Ghana, Legon, Accra, Ghana, [4]Department of Biochemistry and Forensic Sciences, School of Chemical and Biochemical Sciences, University of Technology and Applied Sciences, Navrongo, Ghana

Malaria infection caused by *Plasmodium falciparum* and helminths infections are of significant health concerns. Despite the deployment of malaria vector control and the annual Mass Drug Administration by National Control Programmes, these infections still pose major public health concerns in Ghana. Adequate data is necessary for formulating policies and strengthening interventions to mitigate transmission. This study determined the prevalence of *P. falciparum* and helminths among school children in Saforo and Vunania Primary Schools in the Kassena-Nankana Municipality of the Upper East Region of Ghana. In a cross-sectional survey, capillary blood and stool samples were collected from 120 participants and examined for malaria parasites and helminths presence using microscopy. Structured questionnaire was administered to participants to assess their knowledge, attitudes and practices towards malaria and helminths infections. The overall prevalence of *P. falciparum* in the study was 28.3%; 31.3% in Saforo primary and 24.5% in Vunania primary with females having higher prevalence in both schools (19.4% vs 15.1%, $P = 0.411$ in Saforo and Vunania, respectively). There was no helminths infections reported among the school children. The school children had good (90%) knowledge about both malaria and helminths infections. There was a significant difference ($P<0.001$) between participants' knowledge, attitudes and practices towards malaria. Knowledge and attitude towards helminths infection were good (93%) but participants had poor practices towards helminths infections. Knowledge on the prevalence of infection will serve as a guide in developing sound integrated intervention strategies to reduce the burden of malaria and helminths infections.

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CANNABIDIOL ALTERS ERYTHROCYTE SUSCEPTIBILITY TO *PLASMODIUM FALCIPARUM* INVASION AND MODULATES OSMOTIC FRAGILITY

Eduardo Fernandez Rivadeneira[1], Agostina Marquez[2], Cristina Ramirez[3], Cybele García[2], Cora Alvarez[4]
[1]Institute of Biological Chemistry and Physical Chemistry (IQUIFIB), Ciudad Autónoma de Buenos Aires, Argentina, [2]Faculty of Exact and Natural Sciences, Virology Laboratory, Department of Biological Chemistry, University of Buenos Aires; Institute of Biological Chemistry of the Faculty of Exact and Natural Sciences (IQUIBICEN), Ciudad Autónoma de Buenos Aires, Argentina, [3]Faculty of Exact and Natural Sciences, National University of Mar del Plata; CBG2000 Civil Association, Mar Del Plata, Argentina, [4]Institute of Biological Chemistry and Physical Chemistry (IQUIFIB); Faculty of Exact and Natural Sciences, Biodiversity and Experimental Biology, University of Buenos Aires, Ciudad Autónoma de Buenos Aires, Argentina

Plasmodium falciparum, the causative agent of severe malaria, invades red blood cells (RBCs), leading to hemolysis, microvascular obstruction, and cytoadherence of infected RBCs (iRBCs). These processes are associated with metabolic and structural alterations in both infected and uninfected RBCs (uiRBCs). Cannabidiol (CBD), a major non-psychoactive cannabinoid derived from *Cannabis sativa*, has anti-inflammatory, antioxidant, and reported mild antimalarial properties. We hypothesized that

CBD modulates RBC membrane properties, affecting merozoite invasion in a time-dependent manner. This study evaluated RBC invasion and osmotic fragility in infected and uninfected RBCs in the presence of CBD. The *Plasmodium falciparum* FCR3 strain was maintained in A+ human RBCs (3-5% hematocrit) in supplemented RPMI 1640 medium containing 1% Albumax II under controlled atmosphere (90% N_2 / 5% O_2 / 5% CO_2, pH 7.4) at 37°C. Trophozoite-stage iRBCs were incubated with CBD (10 µM) or vehicle for 1 hour, or CBD was maintained throughout 24 hours. Parasitemia was assessed by Giemsa-stained blood smears at baseline and after 24 hours.During incubation, trophozoite-stage iRBCs matured, ruptured, and released invasive merozoites that subsequently appeared as ring-stage parasites in newly infected RBCs. This process was not affected by a 1-hour CBD treatment. However, when CBD was present throughout the entire cycle, parasitemia was reduced by approximately 80%. Moreover, pretreatment of control RBCs with CBD for 24 hours reduced subsequent infection by 70% compared to vehicle-treated cells. Osmotic fragility assays revealed a leftward shift in the hemolysis curve of CBD-treated iRBCs, suggesting increased resistance to osmotic lysis. This effect was not observed in uiRBCs. Continuous presence of CBD during invasion is required to significantly reduce parasite propagation and may protect infected RBCs from osmotic damage through modulation of membrane biophysical properties.

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HIDDEN IN THE HOST: EVIDENCE FOR INCREASED SEQUESTRATION IN PF7G8 COMPARED TO PFNF54 IN MALARIA-NAÏVE HUMAN SUBJECTS

Dianna E.B. Hergott[1], Weston Staubus[1], Melanie J. Shears[1], Annette M. Seilie[2], Ashley Subijanto[3], Stephen L. Hoffman[4], B. Kim Lee Sim[5], James G. Kublin[6], **Sean C. Murphy**[3]

[1]University of Washington, Seattle, WA, United States, [2]University of Washingon, Seattle, WA, United States, [3]Univ of Washington, Seattle, WA, United States, [4]Sanaria Inc, Rockville, MD, United States, [5]Sanaria Inc., Rockville, MD, United States, [6]Fred Hutch, Seattle, WA, United States

Sequestration by adherence of parasitized erythrocytes to endothelial cells is a characteristic behavior of *Plasmodium falciparum* (Pf). Several in vitro studies have indicated that two strains commonly used in vaccine development and controlled human malaria infection (CHMI), PfN54 and Pf7G8, may sequester to varying degrees. CHMI trials with these strains offer an opportunity to study this phenomenon in vivo. We analyzed the kinetics of early blood-stage infections in 71 participants across seven CHMI trials conducted in Seattle, WA between 2010 and 2022. Participants underwent CHMI with either PfNF54 (n=60) or Pf7G8 (n=11) and were tested daily with a highly sensitive *Plasmodium* 18S rRNA qRT-PCR assay with a limit of detection of ~10 parasites/mL of blood. We used binomial mixed-effects regression to model the odds that a participant would have a lower parasite density (any sequestration) 24 hours after their first positive test result by strain, adjusting for initial density and sex, with study as a random effect. We then looked at the odds that a participant would have undetectable parasites 24 hours after first positive (strong sequestration) using the same model. Parasite density at first detection was similar between PfNF54 (6.11 ± 0.60 $\log_{10}$ copies/mL) and Pf7G8 (6.26 ± 0.52 $\log_{10}$ copies/mL; p=0.33). However, Pf7G8 was associated with significantly higher adjusted odds of any sequestration (OR: 19.64; 95% CI: 3.71-103.9) and strong sequestration (OR: 11.40; 95% CI: 1.96-66.46) following first detectable parasitemia. In line with in vitro findings, our results demonstrate that Pf7G8 exhibits a more pronounced sequestration phenotype than PfNF54 in vivo. Understanding strain-specific biological differences can help guide optimal diagnostic or treatment guidelines during CHMI studies.

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ROLE OF LYSINE DEMETHYLATION AND LACTYLATION IN EPIGENETIC REGULATION OF *PLASMODIUM FALCIPARUM* GAMETOCYTES

Isaac Owusu-Frimpong[1], Abhai K. Tripathi[2], Vladimir Shulaev[3], David J. Sullivan[4]

[1]Johns Hopkins Sch of Public Health, Dept Molecular Microbiology & Immunology, Baltimore, MD, United States, [2]Johns Hopkins Malaria Research Institute, Baltimore, MD, United States, [3]University of North Texas, Denton, TX, United States, [4]John Hopkins Univ, Baltimore, MD, United States

Gametocytes represent a radical critical departure from the rapidly replicating asexual intraerythrocytic *Plasmodium*. The transcription factor *Pf*AP2-G is the master regulator of the coordinated transition to gametocyte generation. Numerous environments have been associated with an apparent higher *Plasmodium* conversion rate from asexual stages to gametocytes, like an elevated lactate, reticulocyte-rich environment, parasite-conditioned media, drug-induced stress, extracellular vesicles, and reduced lysophosphatidylcholine (LysoPC) level in culture media. Lyso PC levels below 5 µM (from typical plasma levels near 100 µM) reduce histone methylation-mediated repression of *Pf*AP2-G and promote gametocyte commitment. We observed increased quantity of gametocytes when daily culture media changes were supplemented with 5 mM racemic lactate in five separate 16-day gametocyte trials. The increased quality of gametocytes was demonstrated with increased mosquito oocyst prevalence and total oocyst numbers. We explored histone lysine lactylation, a recently described additional novel epigenetic modification, as a mechanistic link between high lactate and gametocyte commitment. We hypothesize a slower histone lysine lactylation off-rate compared to lysine acetylation off-rate, which epigenetically keeps chromatin open, triggering PfAP2-G after lower LysoPC or elevated lactate levels lead to histone demethylation. L-lactate is potent below 5 mM for gametocyte induction. We detected a subset of lactylated histone-related proteins by mass spectrometric analysis, including 1) histone H2A; 2) histone-arginine methyltransferase CARM1; 3) lysine-specific histone demethylase 1; 4) histone acetyltransferase GCN5; 5) histone-lysine N-methyltransferase SET2; and 6) histone deacetylase 2. This work mechanistically characterizes the role of lactylation in an open chromatin structure, which increases opportunities for the induction of *Pf*AP2-G.

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DIFFERENTIAL GENE EXPRESSION IN *PLASMODIUM FALCIPARUM* PARASITES GROWN IN PHYSIOLOGICALLY RELEVANT NORMOXIC AND HYPOXIC CONDITIONS *IN VITRO*

Kevin A. Coffey[1], James B. Pease[2], Amy Kristine Bei[3], Regina Joice Cordy[1]

[1]Wake Forest University, Winston Salem, NC, United States, [2]The Ohio State University, Columbus, OH, United States, [3]Yale School of Public Health, New Haven, CT, United States

Plasmodium falciparum has the ability to adapt to diverse oxygen (O_2) levels during its complex lifecycle. While infecting the *Anopheline* mosquito, the parasite is exposed to atmospheric levels of O_2 (~21 O_2). When infecting the human host, the parasite is exposed to a range of O_2 levels (1% to 14%) depending on the anatomical niche it occupies during infection. During the in vitro intraerythrocytic development cycle, the parasite is a microaerophile and replicates best under hypoxic conditions (0.5 to 5% O_2), but can still grow in higher O_2 concentrations. Here, we conducted a longitudinal study using Illumina bulk RNA-sequencing to investigate gene expression patterns of *P. falciparum* grown under hypoxic (1% O_2) versus normoxic (13% O_2) conditions over a 96-hour period, with samples collected at 0, 2, 4, 8, 48, and 96 hours. Differential expression was performed using DESeq2, followed by Gene Ontology (GO) enrichment analyses on PlasmoDB. No significant transcriptional differences were observed from the beginning of O_2 treatment to 8 hours, with a Bonferroni-corrected P-value <0.01, but

significantly differentially expressed genes (DEGs) were found at the 48 and 96-hour timepoints post-O_2 treatment, with the most DEGs observed at the 96-hour timepoint. Using GO enrichment analysis, we identified DNA repair, ATP-dependent activity, and oxidoreductase activity as significantly enriched pathways among the DEGs elevated in hypoxic cultures. Cell adhesion, cell motility, ribosome biogenesis, and mRNA binding were significantly enriched pathways in cultures grown under normoxia. These results suggest that *P. falciparum* adapts to physiological oxygen levels encountered in the body by altering gene expression. Our lab is pursuing ongoing studies to validate these findings and investigate the mechanism(s) underlying the parasite's observed difference in growth rate between hypoxic and normoxic environments.

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PVDBP1 COPY NUMBER VARIATION MODULATES TRANSCRIPT ABUNDANCE AND IS ASSOCIATED WITH NATURALLY ACQUIRED ANTI-PVDBP ANTIBODIES IN CLINICAL *PLAMODIUM VIVAX* INFECTIONS

Nontokozo Mdluli-Berndt[1], Bernis Neneyoh Yengo[1], Meshesha Tsigie[2], Amy Ott[1], James Burns[1], Christopher King[3], Abnet Abebe Assefa[2], Jean Popovici[4], Eugenia Lo[1]

[1]Drexel University College of Medicine, Philadelphia, PA, United States, [2]Ethiopian Public Health Institute, Addis Ababa, Ethiopia, [3]Case Western Reserve University, Moreland Hills, OH, United States, [4]Institut Pasteur of Cambodia, Phnom Penh, Cambodia

Plamodium vivax (Pv) relies on the Duffy Binding Protein (PvDBP1) for reticulocyte invasion, making it a key target of inhibitory antibodies that drive parasite adaptation. Although PvDBP1 expansion has been shown to provide Pv protection from antibody neutralization in vitro, it remains unclear whether naturally acquired antibodies are associated with the distribution of multicopy PvDBP1 parasites. Here, we examined the prevalence, transcriptional, and immunological associations of PvDBP1 copy number variation (CNV) across epidemiologically distinct Pv transmission settings in Ethiopia. In a cross-sectional study of 211 clinical Pv infections from Pv-dominant and Pf-dominant (Bonga) regions, multi-PvDBP1 copy parasites predominate (80%) across sites and host Duffy genotypes, indicating that multicopy PvDBP1 parasites are not restricted to specific epidemiological settings or host backgrounds but widely circulate in malaria endemic regions. Increased copy number was associated with elevated in vivo PvDBP1 transcript levels independent of parasite load and multiplicity of infection, supporting a gene dosage effect that may increase invasion ligand abundance. Despite this increase in PvDBP1 expression, transcript levels were not correlated with anti-PvDBPII IgG magnitude. In some transmission settings, infections harboring multi-PvDBP1 copies had increased odds of high anti-PvDBPII IgG responses, suggesting that PvDBP1 expansion may allow parasites to better adapt and persist under elevated humoral immune pressure. These results highlight a potential mechanism by which Pv modulates PvDBP1 expression to tolerate inhibitory antibody responses, potentially reducing the effectiveness of DBP-based vaccines intended to boost anti-DBP humoral immunity. Further studies are needed to assess the persistence of multicopy infections across varying immune contexts including asymptomatic infections as well as in the context of binding-inhibitory antibodies, to understand how antigen expression relates to antibody function.

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TARGETED DRUG ADMINISTRATION INTERRUPTS MALARIA TRANSMISSION IN BORDER COMMUNITIES OF COSTA RICA

Jennyffer Gonzalez[1], Hazel Herra[1], Rodrigo Marin[1], Isaac Vargas[1], Yadel Centeno[2], Elena Rodriguez[2], Sara Arce[2], Emma Margarita Iriarte[3], Leopoldo Villegas[4]

[1]Ministry of Health, San Jose, Costa Rica, [2]Consultant IREM, San Jose, Costa Rica, [3]Inter American Development Bank-IDB, Washington, DC, United States, [4]International Advisor Independent, Silver Spring, MD, United States

Residual malaria transmission along the Costa Rica-Nicaragua border is driven by mobility and focal outbreaks. In 2023, targeted drug administration (TDA) with chloroquine was implemented in three high-risk communities (Medio Queso, Coquital, San Gerardo) following a *Plamodium vivax* outbreak. A programmatic evaluation using national surveillance data (2021-2025) assessed malaria incidence, locally acquired infections, and transmission clusters at 6, 12, 24, and 36 months post-intervention. TDA achieved 93.3% coverage (4,316/4,624 individuals), with 68.1% completing treatment. Following implementation, autochthonous malaria cases declined to zero within one month and remained at zero at 6, 12, 24, and 36 months of follow-up. This represents a 100% reduction in local transmission, indicating complete interruption of onward transmission. Surveillance has been sustained to detect potential reintroduction. Targeted drug administration implemented during a focal outbreak resulted in rapid interruption and sustained absence of transmission. When integrated with strengthened surveillance and cross-border coordination, TDA represents a high-impact strategy to accelerate malaria elimination in low-transmission settings.

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ENHANCING SEASONAL MALARIA CHEMOPREVENTION PERFORMANCE USING A DATA-INFORMED DECISION-MAKING (DIDM) TOOL: EVIDENCE FROM END-OF-CYCLE SURVEY INDICATORS IN OYO STATE, NIGERIA

Ayodeji Joseph Balogun[1], Taiwo Iyabode Olarinde[1], Moses Enemona John[1], Catherine Ojiro Onoriede[1], Jennifer Ugochi Chukwumerije[2], Oguoma Chibuzo Oguoma[2], Olabisi Adebo Ogunmola[2], Bronwen Davies[3], Jega Ridwan[4]

[1]Malaria Consortium, Ogbomosho, Nigeria, [2]Malaria Consortium, FCT, Nigeria, [3]Malaria Consortium, Cambridge, London, United Kingdom, [4]Malaria Consortium, Sokoto, Nigeria

Seasonal Malaria Chemoprevention (SMC) is a high impact intervention to prevent malaria among children aged 3-59 months through administration of sulfadoxine-pyrimethamine and amodiaquine (SPAQ) during peak transmission. As SMC implementation has expanded in Nigeria, maintaining program quality and data integrity has become increasingly important. To strengthen monitoring and supervision, a Data Informed Decision-Making (DiDM) hypothesis-testing tool using Lot Quality Assurance Sampling (LQAS) aligned End-of-Cycle (EoC) survey data was implemented. Evidence on whether this approach improves SMC delivery remains limited. This study assessed changes in key SMC implementation indicators after introduction of the DiDM tool during the 2025 SMC round in Oyo State. A repeated cross-sectional analysis was conducted using EoC survey data from four SMC cycles in six Local Government Areas. A total of 6,000 caregiver-child pairs were sampled using LQAS (1,500 per cycle). Data were entered into the DiDM tool, which automated interpretation, flagged underperforming indicators and generated actionable recommendations. Primary outcomes included household visitation, SMC coverage among eligible children, direct observation of Day 1 SPAQ administration, and completion of Day 2 and 3 doses. Data were analysed using Stata 16, and chi-square tests assessed differences across cycles at the 95% confidence level. Following introduction of the DiDM tool, household visitation improved significantly from 97.7% in Cycle 1 to 99.5% in Cycle 3 with a slight decline to 98.6% in Cycle 4 ($p < 0.05$). SMC coverage rose from 96.2% in Cycle

1 to 98.7% in Cycle 3 and remained high in Cycle 4 ($p < 0.05$). Direct observation of Day 1 SPAQ administration improved from 93.6% in Cycle 1 to 96.3% in Cycle 4 ($p < 0.05$). Completion of the three-day SPAQ regimen improved from 97.0% in Cycle 1 to 98.7% in Cycle 4 ($p < 0.05$). DiDM-supported EoC surveys were associated with significant improvements in SMC implementation indicators by enabling real-time monitoring, timely feedback, and targeted corrective actions to support data-driven decision making in large-scale SMC programs.

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ROUTINE SEASONAL MALARIA CHEMOPREVENTION (SMC) PILOT STUDY IN MALI

Alassane Bangoura[1], Mady Cissoko[2], Cheikh Sidiya Gassama[1], Keita Fatoumata[1], Jeanbaptiste Kamate[1], Boubacar Sidiki Maiga[1], Famakan Kane[3], Aissata Kone[2], Suzanne Van Hulle[4], Yameni Chrestien[5]

[1]Catholic Relief Services - CRS, Bamako, Mali, [2]National Malaria Control Program - Mali, Bamako, Mali, [3]Health district, Bamako, Mali, [4]Catholic Relief Services, Baltimore, MD, United States, [5]Catholic Relief Services, Dakar, Senegal

Seasonal Malaria Chemoprevention (SMC) was introduced in Mali in 2012 as a complementary preventive measure for children aged 3 to 59 months. Administered in 3 to 5 cycles depending on seasonality, the campaign-based model utilizes a door-to-door strategy led by community health workers over a five-day period. However, this model faces challenges such as high logistical costs and a lack of community engagement. To ensure sustainability and effectiveness, a pilot phase for routine SMC implementation was proposed. This strategy includes social mobilization through advocacy with local authorities, feedback sessions, selection of community health workers (CHWs), and orientation of stakeholders on routine delivery of SMC. This is followed by the provision of Sulfadoxine-Pyrimethamine + Amodiaquine drugs, tools and supplies, and formative supervision. Under this model, parents bring their children to health centers to receive the medication over three days. If a dose is missed, a CHW visits the home to administer it. The study was implemented in two rural health areas within the Bla health district, Ségou region. Sites were selected based on community engagement and commitment of the Chief Medical Officer, with the involvement of Technical Directors (DTC). Data collection was digitized using smartphones, and data were extracted from the national DHIS2 system. The analysis presented data across four cycles in the two health areas. The study covered 9 villages and targeted 3,780 children over 4 months. Coverage across all four cycles was higher in NGolokouna than in Beguene. Coverage in Cycle 1 (73.29%) and Cycle 4 (81.58%) remained higher than in Cycle 2 (55.66%) and Cycle 3 (47.96%), resulting in an overall coverage of 64.62%. A slight increase was noted among children aged 12-59 months compared to those aged 3-11 months. The results demonstrate that delivering SMC through a routine model does not compromise the coverage achieved through a campaign-style model. Nevertheless, a monitoring mechanism must be established to ensure complete data entry and improve coverage across all four cycles before scaling up the program to other health districts.

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SOCIO-DEMOGRAPHIC FACTORS INFLUENCING UPTAKE OF SEASONAL MALARIA CHEMOPREVENTION IN KADUNA STATE, NIGERIA

Obinna Anthony Chukwuzoba[1], Pauline Ukamaka Umeanaeto[1], Sule Danga[2]

[1]Nnamdi Azikiwe University, Awka, Anambra State, Nigeria, [2]State Ministry of Health, Kaduna, Kaduna State, Nigeria

Seasonal Malaria Chemoprevention (SMC) is a proven intervention for preventing malaria among children aged 3-59 months in areas with seasonal transmission. In Kaduna State, Nigeria, variations in uptake persist despite widespread implementation. This study examined socio-demographic factors influencing SMC uptake among eligible children. A cross-sectional study was conducted among 586 caregivers in selected wards across eight LGAs in Kaduna State immediately following the 2025 SMC implementation cycle. Data were collected using pretested structured questionnaires capturing socio-demographic characteristics and SMC uptake. Uptake was defined as receipt of the recommended SMC cycles. Data were analyzed using descriptive statistics and logistic regression to identify factors associated with uptake at $P<0.05$. SMC uptake was generally high (95%) but varied across socio-demographic groups. Higher caregiver education was associated with complete uptake. Adherence to dosage completion was higher among married caregivers (92%) than unmarried caregivers (89%). This may be attributed to increased household support and shared decision-making, which facilitate compliance with multi-day SMC dosing. Caregivers with stable occupations and smaller household sizes were more likely to complete the recommended SMC cycles. Awareness of SMC and prior exposure to campaigns also positively influenced uptake, while low education and larger household sizes were linked to incomplete uptake. Socio-demographic factors play an important role in determining SMC uptake in Kaduna State. Strengthening targeted community sensitization, especially among less-educated caregivers and larger households, will be critical to improving equitable coverage and enhancing the overall impact of the program.

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WHO IS LEFT BEHIND? AN EQUITY ANALYSIS OF SEASONAL MALARIA CHEMOPREVENTION UPTAKE IN NIGERIA

Elvis Efe Isere, Olabisi Adebo Ogunmola, Alhassan Alhaji Bulama, Chibuzo Oguoma, Jennifer Ugochi Chukwumerije

Malaria Consortium, Abuja, Nigeria

Seasonal malaria chemoprevention (SMC) delivers preventive antimalarials to children aged 3-59 months in Nigerian states with highly seasonal malaria transmission. As SMC programmes transition from rapid scale-up to sustained intervention and coverage approaches saturation, ensuring equitable reach becomes increasingly critical. Persistent gaps, particularly among urban and socioeconomically disadvantaged populations, may undermine progress and widen disparities among SMC eligible children. We conducted an equity-focused analysis of 2025 end-of-cycle (EoC) SMC data from nine states to identify children at risk of being missed. The EoC survey data, collected using a Lot Quality Assurance Sampling (LQAS) methodology, were analysed. A total of 144,474 caregiver–child records were included. The primary outcome was non receipt of SMC. Equity stratifiers included caregiver literacy, education, occupation, gender, and community type. Bivariate associations were assessed using chi-square tests. Multivariable logistic regression estimated adjusted odds ratios (aORs) with 95% confidence intervals (CIs). Overall non-receipt of SMC was 5.2% (n=7,471). Children of literate caregivers had lower odds of missing SMC (aOR=0.67; 95% CI: 0.63–0.71). Compared with higher education, lower educational attainment was associated with lower odds: informal (aOR=0.55; 95% CI: 0.50–0.62), primary (aOR=0.55; 95% CI: 0.49–0.62), and secondary (aOR=0.65; 95% CI: 0.59–0.71). Unpaid agricultural workers (aOR=0.64; 95% CI: 0.57–0.71) and unemployed caregivers (aOR=0.61; 95% CI: 0.53–0.70) had lower odds. Rural children were less likely to miss SMC than urban children (aOR=0.73; 95% CI: 0.69–0.78). High overall SMC coverage masks persistent urban and socioeconomic inequities. Targeted strategies are needed to enhance SMC access within urban settlements and among socioeconomically disadvantaged eligible children, including routine equity monitoring within SMC performance systems to identify and eliminate these pockets of residual exclusion.

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TECH ENABLED LARVAL SOURCE MANAGEMENT FOR MALARIA CONTROL IN KIGALI, RWANDA: UAV LARVAL HABITAT TARGETING WITH AI MODELING

Emmanuel Hakizimana[1], Dunia Munyakanage[1], Felix Ndahimana[2], Eric Remera[1], Teddy Segore[3], Brenda Mutamba Karemera[4], Jean Baptiste Ukwizagira[4], Innocent Ndikumana[5], Alexis Rulisa[6], Eric Rutayisire[3], Edward Ned Walker[7], Jackie Cook[8], Charles Bucagu[2], Chantal Ingabire[2], Aimable Mbituyumuremyi[1], Mamy Muziga Ingabire[4]

[1]Ministry of Health/Rwanda Biomedical Center, Kigali, Rwanda, [2]University of Rwanda, Kigali, Rwanda, [3]Charis UAS, Kigali, Rwanda, [4]Tech for Health Equity Foundation, Kigali, Rwanda, [5]Rwanda Agricultural Board, Kigali, Rwanda, [6]University, Kigali, Rwanda, [7]Michigan State University, East Lansing, Michigan, MI, United States, [8]London School for Hygiene and Tropical Medicine, London, United Kingdom

Larval source management (LSM) expands the arsenal of malaria vector control tools. The efficiency of LSM can be enhanced by integration of technologies such as drone-based imaging of landscapes, artificial intelligence, and focused larvicide applications by Unmanned Aerial Vehicles (UAVs). Our research develops an AI-informed model for a targeted LSM intervention with synergy with agricultural activity and community engagement. The three-year program is positioned in mostly modified marshlands of peri-urban Kigali, Rwanda, an area of high-density human settlements, juxtaposed to irrigated rice farming, row crop production, and extraction of clay and sand for constructions. These activities create habitats for *Anopheles* larval breeding. Human landing catches of adult *Anopheles* vectors, primarily *Anopheles arabiensis*, revealed higher vector densities in areas closest to these lowlands while clinic-based surveys revealed higher malaria incidence. The study design invokes a mixed-methods approach intertwining the following five themes: **first**, remote sensing of landscape and land cover using UAVs with AI-trained habitat detection furthers an AI-enabled LSM system. The system is centered in an AI-model trained from entomological sampling and image analysis. This model will be evaluated and validated for effective, targeted LSM in the succeeding two years of work. **Second**, entomological and epidemiological assessments during the implementation and validation phases measure the impact of targeted LSM on larval and adult mosquito abundance, mosquito biting rate, and malaria case incidence. **Third**, agricultural experiments measure the impact of targeted LSM on rice productivity and attendant farming practices. **Fourth**, the operational and programmatic efficiency and cost effectiveness invoke multi-dimensional economic evaluation. The **fifth** set of activities focuses on community engagement, perceptions and socio-economic impact. Overall, the project will generate strong evidences on key metrics of LSM and thus enable scalable and cost effective LSM intervention in similar settings of malaria transmission.

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TRENDS AND INEQUALITIES IN THE UPTAKE OF AT LEAST THREE DOSES OF INTERMITTENT PREVENTIVE TREATMENT (IPTP3$^+$) AMONG PREGNANT WOMEN IN GHANA, 2003-2022

Martin Badagda Lugutuah[1], Julius Kwabena Karikari[2], Kweku Bedu-Addo[3], Emmanuel Konadu[2], Douglas Aninng Opoku[2], Yahye Sheikh Abdulle Hassan[4], Linda Asare-Adjebeng Bedu-Addo[2], Eric Adjei Boadu[5]

[1]National Malaria Elimination Programme, Accra, Ghana, [2]University Hospital, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, [3]Department of Anaesthesia and Intensive Care, Komfo Anokye Teaching Hospital, Kumasi, Ghana, [4]Faculty of Medicine and Health Sciences, Jamhuriya University of Science and Technology, Mogadishu, Somalia, [5]Department of Population, Family and Reproductive Health, School of Public Health, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

Intermittent preventive treatment during pregnancy (IPTp) is a key strategy for malaria control recommended by the World Health Organization (WHO). However, in Ghana, uptake of at least three doses (IPTp3$^+$) remains below the WHO target of 80%. This study therefore examined the trends and inequalities in IPTp3$^+$ uptake among pregnant women between 2003 and 2022. This study analyzed data from the 2003, 2008, 2014, and 2022 Ghana Demographic and Health Surveys. The WHO Health Equity Assessment Toolkit was used to assess trends and inequalities in IPTp3$^+$ uptake across wealth quintiles, educational attainment, place of residence, and subnational regions. Inequalities were quantified using absolute measures [Difference (D) and Population Attributable Risk (PAR)] and relative measures [Ratio (R) and Population Attributable Fraction (PAF)]. National IPTp3$^+$ uptake increased markedly from 0.8% in 2003 to 60.2% in 2022. Regional inequalities were the largest, widening from D = 2.0; PAF = 138.3; PAR = 1.2 in 2003 to D = 34.4; R = 1.8; PAF = 30.8; PAR = 18.6 in 2022. Economic disparities were minimal in 2003 (D = -0.7; R = 0.5; PAF = 0.0; PAR = 0.0) but grew substantially in 2008 (D = 18.0; R = 2.0; PAF = 31.3; PAR = 8.8) and persisted in 2022 (D = 14.0; R = 1.3; PAF = 13.4; PAR = 8.1). Educational inequalities were absent in 2003 but peaked in 2008 (D = 29.0; R = 2.4; PAF = 78.2; PAR = 21.9) before narrowing by 2022 (D = 12.5; R = 1.2; PAF = 7.7; PAR = 4.7). Urban-rural differences remained modest across the study period, with disparities of D = -0.1; R = 0.9; PAF = 0.0; PAR = 0.0 in 2003 and D = 4.8; R = 1.1; PAF = 4.3; PAR = 2.6 in 2022. Ghana has made significant progress in scaling up IPTp3$^+$ coverage over the past two decades. However, persistent regional, economic, and educational disparities threaten equitable access to malaria prevention. Targeted, equity-focused interventions, particularly in underserved regions and among socioeconomically disadvantaged women, are critical for achieving universal IPTp3$^+$ coverage and improving maternal and neonatal health outcomes.

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INTEGRATING SEASONAL MALARIA CHEMOPREVENTION WITH MALARIA VACCINATION: LESSONS LEARNED FROM THE GAOUAL HEALTH DISTRICT, GUINEA

Mamadou Sitan Keita[1], Sekouba Cisse[1], Souleymane Berete[1], Daouda N'diaye[1], Fakouma Camara[1], Mohamed Saran Conde[1], Hamidou 2 Barry[2], Abdourahamane Diallo[3], Tahirou Diallo[4], Bintou Conde[5], Jeremi Leno[4], Hawa Bah[6], Albert Camara[7], Daman Keita[7], Alioune Camara[3]

[1]Catholic Relief Services, Conakry, Guinea, [2]ChildFund International, Mamou, Guinea, [3]National Malaria Control Program, Conakry, Guinea, [4]Prefectural Health Directorate, Gaoual, Guinea, [5]Prefectural Health Directorate, Dubreka, Guinea, [6]IQVIA, Conakry, Guinea, [7]Expanded Programme on Immunization, Conakry, Guinea

In the context of the first year of malaria vaccine introduction in Guinea, the Gaoual health district implemented an innovative approach integrating seasonal malaria chemoprevention (SMC) with malaria vaccination to improve access to and coverage of both interventions among children. An integrated SMC-malaria vaccination strategy was implemented during the third and fourth SMC cycles (August-September 2025), following a preparatory phase that included planning, advocacy, and community mobilization. Doortodoor SMC distribution teams systematically identified and referred eligible children to health facilities using referral forms, where dedicated vaccinators provided malaria vaccination. Children who were referred but did not present to health facilities were actively traced by community health workers. Vaccination data were collected daily and validated at the district level. Malaria vaccination was launched on August 13, 2025. Over a 49day period (August 13-September 30, 2025), a total of 2,334 eligible children received the first dose of the malaria vaccine, of whom 973 (42%) were vaccinated during the eight cumulative days of the SMC campaign. Similarly, among the 687 children who received the second dose, 325 (47%) were vaccinated during SMC campaign days, highlighting the substantial contribution of the integrated approach to the rapid increase in vaccination coverage. The integration of SMC with malaria vaccination proved to be an effective and equitable strategy to accelerate vaccine coverage during the introduction phase. Despite some operational

constraints, this integrated approach represents a relevant programmatic option for strengthening malaria prevention and could be replicated in similar settings.

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OPTIMISING SEASONAL MALARIA CHEMOPREVENTION IN MALAWI: A MODELLING STUDY OF DRUG REGIMENS AND TIMING

Donnie Mategula[1], Alinafe Maenje[2], Chisomo Mkwanda[2], Steven Munharo[2], Lumbani Munthali[3], Lauren Cohee[4]

[1]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [2]Malawi-Liverpool-Wellcome Programme, Blantyre, Malawi, [3]National Malaria Control Programme, Lilongwe, Malawi, [4]Center for Vaccine Development and Global Health, University of Maryland SOM, Hillsborough, NC, United States

Seasonal malaria chemoprevention (SMC) is a highly effective strategy that has protected millions of children in the Sahel by significantly reducing clinical malaria and hospitalisation during peak transmission periods. The 2022 World Health Organisation recommendation to expand SMC beyond the Sahel created an important opportunity to extend access to this intervention to other malaria-endemic areas. In Malawi, malaria transmission is highly seasonal, primarily occurring from November to May. However, the likely effectiveness of SMC in East and Southern Africa remains uncertain due to differences in transmission patterns and antimalarial drug resistance. We developed a mathematical malaria transmission model to assess the impact of chemoprevention in Malawi. The model was calibrated separately for 16 districts using routine malaria case data and climate variables to reflect local transmission dynamics. Districts where malaria vaccination had been introduced were excluded because the model did not perform well in the presence of vaccine effects. We simulated five monthly treatment cycles, aligned with Malawi's malaria season, starting in December, January, or February. Regimens assessed included dihydroartemisinin-piperaquine (DP), sulfadoxine-pyrimethamine (SP), amodiaquine (AQ), sulfadoxine-pyrimethamine plus amodiaquine (SPAQ), chloroquine (CQ), and artemether-lumefantrine (AL). Projected efficacy varied by district, regimen, and timing. DP and SPAQ showed the highest projected efficacy, exceeding 70% in Dowa, Kasungu, Mwanza, and Mzimba. Initiating treatment earlier led to greater reductions in predicted malaria burden among children under five years of age than later start dates. Chemoprevention could offer substantial benefits in Malawi, but its impact is likely influenced by local epidemiology, drug selection, and timing of implementation. DP and SPAQ emerged as the most promising regimens based on this modelling analysis. These findings provide policy-relevant evidence to guide the adaptation of SMC strategies in Malawi and other settings in East and Southern Africa.

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HANDLING THE STORM: REALITIES OF IMPLEMENTATION AND MODIFICATIONS DURING LIBERIA'S NATIONAL MALARIA STRATEGIC PLAN 2021-2025 END-TERM REVIEW

Victor S. Koko[1], Trokon Washington[2], Joseph O. Alade[3], Jethro Zawolo[4], Yah M. Zolia[5], Uwem Inyang[6]

[1]Ministry of Health, National Malaria Control Program, 1000-Monrovia, 10, Liberia, [2]National Malaria Control Program, Monrovia, Liberia, [3]National Malaria Control, 675, Liberia, [4]DoS TA, 1000-Monrovia, 10, Liberia, [5]Consultant, 1000-Monrovia, 10, Liberia, [6]Consultant, Monrovia, Liberia

The National Malaria Control Program (NMCP) conducted its Program Review (MPR) of Liberia's 2021-2025 Strategic Plan in 2025 amidst a challenging global health funding landscape, requiring significant modifications to the traditional review approach. Using the WHO 2020 MPR manual, NMCP adapted a four-phase approach: planning, thematic desk review, validation, and program strengthening due to financial constraints. Adaptations included a cloud-based repository, virtual coordination meetings with WHO, and an MPR secretariat. Seven NMCP-led thematic groups conducted desk reviews. External validation involved field visits to five regions, with two selected for in-depth assessment, alongside national-level stakeholder interviews. Despite extended planning and reduced partner involvement, the review was completed via virtual collaboration and streamlined desk reviews. Malaria prevalence declined 61% between 2016 and 2022 (45% to 17.7%). However, HMIS data showed gains in confirmed and severe malaria cases from 2021-2023 were not sustained, with cases rising in 2024-2025. Entomological surveillance confirmed widespread pyrethroid resistance, enabling transition to dual-active ingredient nets in 2021, though surveillance activities were reduced in 2024-2025 due to funding gaps. In conclusion, The MPR demonstrated NMCP resilience in generating evidence despite funding constraints but highlighted health system vulnerabilities to shifts in global financing. While long-term indicators improved, the current plateau in cases underscores service delivery challenges. Future priorities include mobilizing domestic resources, financial contingency measures, sustainable surveillance platforms, health system resilience, and protocols for adapting implementation during budgetary constraints

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MALARIA PARASITE POSITIVITY RATES AMONG SYMPTOMATIC INDIVIDUALS IN THE NEW JUABEN SOUTH MUNICIPALITY, EASTERN REGION OF GHANA (2023-2025)

Eunice Abuaku[1], Richard Twum[1], Joseph Abankwa[1], Benjamin Kwaku Abuaku[2]

[1]New Juaben South Municipal Health Directorate, Ghana Health Service, Koforidua, Ghana, [2]Epidemiology Department, Noguchi Memorial Institute for Medical Research, Legon, Ghana

Malaria remains one of the major public health concerns in Ghana. In 2023 a malaria elimination strategy was launched in the country drawing lessons from countries that had eliminated malaria. The strategy stratifies the country into four operational zones: districts with high malaria burden (incidence ≥ 500 cases per 1,000 population, parasite prevalence ≥ 15%, and under five mortality ≥ 70); districts with moderate malaria burden (incidence 200-499 cases per 1,000 population, parasite prevalence 5% - < 15%, and under five mortality 60 - < 70); districts with low malaria burden (incidence 50-199 cases per 1,000 population, parasite prevalence 1% - < 5%, and under five mortality 50 - <60) and districts with very low malaria burden (incidence 1-49 cases per 1,000 population, parasite prevalence < 1%, and under five mortality 0 - <50). The New Juaben South Municipality falls within the high malaria burden stratum. Analysis of data obtained since the launch of the malaria elimination strategy show a significant decline of parasite prevalence from 30.1% (95% CI: 29.8 - 30.4) in 2023 to 22.6% (95% CI: 22.3 - 22.9) in 2025 (p=0.000). Sustaining gains made within the municipality will contribute positively towards the national malaria elimination agenda.

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LEVERAGING SATELLITE IMAGERY AND OPEN SOURCE BUILDING FOOTPRINT POLYGONS TO ESTIMATE TARGET POPULATIONS AND SURVEY SAMPLING FRAMEWORKS IN SETTINGS WITHOUT RELIABLE CENSUS DATA

David Galick[1], Jordan Smith[2], Duncan McFarland[2], Carlos Alberto Guerra[3], Guillermo A. Garcia[2]

[1]MCD Global Health, Malabo, Equatorial Guinea, [2]MCD Global Health, Hallowell, ME, United States, [3]MCD Global Health, Quito, Ecuador

Conducting representative population-based surveys requires knowing how many households exist and where they are located, information that is frequently unavailable or unreliable in settings with outdated, incomplete or low spatial resolution census data. We describe a methodology developed by the Bioko Island Malaria Elimination Project (BIMEP) to build a sampling framework for a malaria indicator survey (MIS) in the Continental Region of Equatorial Guinea (EG), covering approximately 680,000 people across 13 districts where no recent household enumeration exists. The approach used satellite imagery for field navigation and spatial reference, freely available building footprint polygons derived from satellite data to estimate the number of households per grid cell, and a spatial decision support system (Campaign Information Management System - CIMS) as

the field platform for identifying households, collecting survey data, and validating household selection. Estimates from the building polygons were adjusted using correction factors for household size, proportion of occupied structures, and average number of dwellings per building, then aligned with district population figures from a census and health system dispensarization methodology. Sampling units were defined from 1km x 1km grid cells, joining or separating such that each unit contained between 100 and 600 households and did not cross district boundaries. Field validation on Annobon Island and in Bata, continental EG, compared estimates to enumerated households on the ground and found acceptable agreement. In dense urban areas, identifying which buildings were homes rather than commercial premises required direct field observation. The resulting sampling framework targeted approximately 3,200 households across 5 provinces. The MIS will be conducted in mid-2026. This approach can be applied wherever reliable population data at sufficiently high resolution are unavailable.

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IMPROVING *PLASMODIUM FALCIPARUM* AMPLICON SEQUENCING THROUGH PCR INHIBITOR REMOVAL

Milya Davlieva[1], Kore Lum[1], Fehintola Ajogbasile[1], Nicholas Hathaway[1], Adrienne Epstein[1], Amy Wesolowski[2], Jean-Bertin Bukasa Kabuya[3], Isabel Rodríguez-Barraquer[1], Jessica Briggs[1], Bryan Greenhouse[1]

[1]Division of HIV, Infectious Diseases, and Global Medicine, Department of Medicine, University of Cal, San Francisco, CA, United States, [2]Johns Hopkins University, Baltimore, MD, United States, [3]Tropical Diseases Research Centre, Ndola, 8, Zambia

Scaling high-resolution molecular surveillance for malaria control and elimination requires genotyping platforms that perform reliably across diverse field conditions. Multiplexed amplicon sequencing is widely used for *Plasmodium falciparum* surveillance but can be sensitive to PCR inhibitors in patient-derived samples, leading to sequencing failure rates ranging from 5% to 95% across studies. To address the lack of systematic approaches to mitigate PCR inhibition, we evaluated strategies on laboratory-derived and field-collected samples, including previously unsuccessful specimens from multiple studies. DNA extracts were processed either directly, after dilution, or treated with the Zymo PCR Inhibitor Removal Kit or Qiagen anti-inhibitor additives. Dilution partially restored amplification in some sample sets, indicating that PCR inhibition is a primary cause of sequencing failure. Zymo-based cleanup reduced median primer dimer rates compared to untreated extracts (2.2% vs 4.9%, $p \leq 0.001$) and improved the median proportion of sequencing reads passing quality filtering (86.6% vs 81.2%, $p \leq 0.05$) for MAD^{4}HatTeR targeted amplicon sequencing. In paired comparisons, Zymo cleanup rescued challenging specimens, achieving an estimated success rate of 80–90% and yielding usable sequencing data even from samples not recoverable by dilution alone. Allele-level comparisons revealed high concordance between methods, with Zymo detecting slightly more low-frequency variants (<1% within-sample allele frequency), while major alleles and drug-resistance classifications remained stable across treatments. Future work will extend these evaluations to additional multiplexed amplicon approaches, including nanopore sequencing and mixed-strain control experiments. These findings highlight PCR inhibition as a critical factor impacting amplicon sequencing performance and support the implementation of inhibitor-removal strategies to enhance the robustness of malaria molecular surveillance workflows.

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FROM DATA TO ACTION: DATADRIVEN MENTORSHIP USING MALARIA CASE MANAGEMENT DASHBOARDS

IniAbasi Nglass[1], Adrian May[1], Elizabeth Lucas Nyakarungu[1], Maria Consuelo Oki Eburi[1], Tony Kazungu[1], Miriam Riobi Patabobe[1], Matilde Riloha Rivas[2], Julie Guadalupe Buekens[3], Wonder Philip Phiri[1], Carlos Alberto Guerra[4], Guillermo A. Garcia[3]

[1]MCD Global Health, Malabo, Equatorial Guinea, [2]National Malaria Control Program, Malabo, Equatorial Guinea, [3]MCD Global Health, Hallowell, ME, United States, [4]MCD Global Health, Quito, Ecuador

Effective use of routine health information data is essential for strengthening malaria case management services; however, these data are often underutilized at the health facility level. Limited access to computers, gaps in digital skills, and difficulties interpreting data and visualizations can hinder the use of data for timely action. The Bioko Island Malaria Elimination Project, in collaboration with the Ministry of Health and the National Malaria Control Program of Equatorial Guinea, integrated the use of data visualizations as part of the Outreach Training and Supportive Supervision (OTSS) approach. In preparation for OTSS visits in December 2025, routine malaria case management data were extracted from the District Health Information System (DHIS2) for three target hospitals in Malabo and Riaba Districts. Key performance indicators—including general attendance, suspected malaria cases, diagnostic tests performed, and confirmed cases treated—were analyzed and visualized before OTSS visits. These data visualizations were printed and served as a primary tool for mentorship discussions between trained supervisors and facility leads. These visualizations prompted structured discussion about facility specific data trends and their implications for case management. Supervisors indicated that these discussions led to an enhanced understanding of malaria case management patterns among facility staff. Gaps identified through this process, combined with digital OTSS checklist results, informed the development of facility-specific improvement plans. This approach directly addresses a common barrier in low-resource settings where DHIS2 data exist but go unused due to limited digital access and data literacy at the facility level. The pilot demonstrated that printed data visualizations can serve as an accessible and practical bridge between routine data systems and frontline decision-making. This integrated approach is now being scaled to all quarterly OTSS visits in 2026, with improvement plans reviewed at each cycle to assess whether data-driven mentorship strengthens facility performance over time.

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GUINEA SALUD ROUND 2: A WHATSAPP-BASED AI CHATBOT FOR STRENGTHENING MALARIA INTERVENTION DELIVERY ON BIOKO ISLAND, EQUATORIAL GUINEA

Alayna Sublette[1], Honorio Borico[2], Duncan McFarland[1], Justino Mba[2], Principe Abraham[2], Monica Aling[2], Matilde Riloha Rivas[3], Wonder Philip Phiri[2], Carlos Alberto Guerra[4], Guillermo A. Garcia[1]

[1]MCD Global Health, Hallowell, ME, United States, [2]MCD Global Health, Malabo, Equatorial Guinea, [3]National Malaria Control Program, Malabo, Equatorial Guinea, [4]MCD Global Health, Quito, Ecuador

Reaching individuals with timely, relevant information about health interventions they have received remains a persistent challenge in settings where Internet access is limited, and populations are dispersed. Leveraging widely used platforms with minimal data usage such as WhatsApp presents an opportunity to strengthen social behavior change communication (SBCC). After one cycle of connecting health intervention data from the Campaign Information Management System (CIMS) to an artificial intelligence (AI) agent on WhatsApp, the Bioko Island Malaria Elimination Project (BIMEP) has identified ways to expand SBCC. While the first cycle of the ChatBot Guinea Salud focused on increasing general knowledge of health interventions, this cycle generates a more robust feedback loop with multiple workflows. Through a series of tailored messages, the AI agent spreads awareness about interventions and suggests actionable steps to improve adherence. This exchange is reciprocal; the user provides

feedback, asks questions, and shares their behaviors. The AI agent responds by sending pre-defined messages that clarify information or direct the user to a relevant resource. Here, we present four workflows: LLIN distribution at fixed pick-up points, LLIN distribution through prenatal care, indoor residual spraying (IRS), and the malaria indicator survey (MIS). The LLIN workflows follow up on net receipt, encourage correct and consistent use, promote LLIN replacement in due time, and capture household location data not available from pick-up point records. The IRS workflow collects qualitative feedback from residents about their experience with spray teams. The MIS workflow sends reminders to individuals who tested positive to complete their treatment. Data generated across all four workflows will provide new behavioral and programmatic indicators to strengthen intervention delivery and inform decision making.

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MOLECULAR SURVEILLANCE OF *PLASMODIUM FALCIPARUM* ANTIMALARIAL MOLECULAR RESISTANCE MARKERS IN NEW YORK STATE PATIENTS FROM 2018 TO 2022

Caitlin Koto[1], Brooke M. Clemons[1], Erica Lasek-Nesselquist[1], Mike Antwi[2], Jennifer White[1], Alissa Collins[1], Susan Madison-Antenucci[1]

[1]New York State Department of Health Wadsworth Center, Albany, NY, United States, [2]NYC Department of Health and Mental Hygiene, Long Island City, NY, United States

From 2018 to 2022, the New York State (NYS) Department of Health, Wadsworth Center Parasitology Laboratory confirmed over 1,200 cases of malaria caused by *Plasmodium falciparum* (*Pf*). Travelers visited over 36 countries with the majority going to West Africa. Epidemiological data indicates that 75% of patients did not take prophylaxis prior to travel. Of the nearly 18% of NYS patients who did report taking prophylaxis, only about 36% of patients self-identified that they took the prophylaxis as prescribed. Mefloquine (Lariam) was the drug prescribed most frequently. Given the number of different countries visited by travelers, detection of *Pf* antimalarial resistance mutations in NYS patients is critical in assisting clinicians to choose proper treatment. In the NYS Parasitology Laboratory, antimalarial resistance mutations are identified through amplicon-based next generation sequencing of six resistance genes, chloroquine resistance transporter, multidrug resistance protein 1, kelch 13, bifunctional dihydrofolate reductase-thymidylate, hydroxymethyl dihydropterin pyrophosphokinase-dihydropteroate synthase, and cytochrome b oxidase (*Pfcrt*, *Pfmdr1, Pfk13, Pfdhfr*, *Pfdhps,* and *Pfcytb,* respectively*)*, which are known to be responsible for drug resistance. Approximately 97% of *Pf* specimens had resistance mutations in both the *Pfdhfr* and *Pfdhps* genes, which are linked to sulfadoxine-pyrimethamine resistance, and about 70% of specimens had mutations in *Pfmdr1*, which is associated with resistance to multiple drugs including quinine, amodiaquine and artemether-lumefantrine. During the five-year period, no *Pf* specimens were identified as having mutations for *Pfk13* or *Pfcytb*, indicating that artemisinin and atovaquone susceptibility likely remains high among returning travelers. By assessing NYS patients with *Pf* malaria through surveillance of molecular markers that cause, or are associated with drug resistance, the NYS Parasitology Laboratory can better assist in providing clinicians and public health specialists with information to provide proper prophylaxis and treatment for travelers to endemic countries.

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UNEVEN PROGRESS TOWARD MALARIA ELIMINATION IN GHANA: A NATIONAL ANALYSIS OF SURVEILLANCE QUALITY AND PROGRAM PERFORMANCE

Ferguson Duvor, Wahjib Mohammed, Hilarius Abiwu

National Malaria Elimination Program, Accra, Ghana

As Ghana advances toward malaria elimination, high-quality surveillance and effective program delivery are essential. Yet comprehensive nationwide assessments of data integrity and program performance remain limited. This study applies a standardized framework to identify gaps and regional disparities.Routine malaria data from DHIMS (January-December 2025) were analyzed using 19 indicators across two domains: data quality (6 indicators) and program performance (13 indicators). Indicators covered reporting completeness, data consistency, testing rates, case burden, mortality, treatment, and preventive interventions (IPTp, LLINs). Each indicator was scored on a five-point scale, with penalties for implausible values. Composite regional scores were converted to percentages for comparison and prioritization.Significant regional variation was observed, with composite scores ranging from 79.8% (Greater Accra) to 32.6% (Northern). High-performing regions demonstrated robust data systems and service delivery, while low-performing regions exhibited weaknesses in data consistency and program outcomes. Malaria burden and institutional mortality were highest in northern regions. Under-five case fatality was alarmingly high across all regions, revealing a critical gap in severe malaria management. Preventive intervention coverage varied, with high IPTp uptake in some areas but widespread dropout and low LLIN use among pregnant women.This assessment reveals marked geographic inequities in surveillance and program performance. The persistently high under-five case fatality signals an urgent need to strengthen severe malaria case management nationally. The framework provides a practical tool for monitoring progress and targeting resources to accelerate elimination.

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EVALUATION OF THE MALARIA SURVEILLANCE SYSTEM: GA EAST MUNICIPALITY, GREATER ACCRA, GHANA, 2023-2024

Abdul M. Falama, Shembla Sayibu, Delia Bandoh, Ernest Kenu, Gebrekrstos N. Gebru

University Of Ghana School Of Public Health, Accra, Ghana

Following substantial malaria burden reduction in Ghana, marked by a national prevalence 8.6% and 21 districts below 5%, Ga East Municipality, with a prevalence of 2.0%, was prioritized for malaria pre-elimination. This transition necessitated evaluation of it's surveillance system. A cross-sectional, mixed-methods evaluation was conducted from April 4th-25th, 2025, guided by the CDC's Updated Guidelines for Evaluating Public Health Surveillance Systems and WHO Malaria Surveillance Toolkit. The evaluation assessed performance, infrastructure, information flow, and key attributes, including usefulness, simplicity, flexibility, data quality, acceptability, representativeness, and stability. Data were collected through facility assessments, stakeholder interviews, and surveillance records review (December 2023-December 2024). Data were analyzed using Microsoft Excel, and the results were presented as frequencies and proportions. Results from the evaluation revealed in 2024, 45,085 suspected malaria cases were reported, of which 44,800 (99.4%) tested, and 12,027 confirmed cases. Test positivity rate was 26.7%, with a crude incidence of 37.4 per 1,000 people. Data reporting processes and information flow shown that 80% of facilities used electronic medical records (EMR) for data capture, summarizing and aggregated data reported into district health information system (DHIMS2), while 20% relied on paper-based and higher-level facility reporting pathways. Health facility participation 40/52 (76%); data completeness and timeliness were 90% and 94.7%, respectively. Surveillance staffs interviewed, 30/30 (100%) perceived the system as simple and flexible, whereas 70% reported stability and acceptability. Although Ga East Municipality's malaria surveillance system functions adequately for malaria control, with complete monthly aggregate reporting, it requires substantial strengthening to meet elimination standards. Current gaps include lack of real-time case-based reporting, limited case investigation capacity, minimal private sector integration, and absence of hotspots geographic mapping.

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DEVELOPMENT AND PRELIMINARY USABILITY TESTING OF VECTOR INSIGHT- AN INTELLIGENT DASHBOARD FOR INTEGRATED MALARIA VECTOR MANAGEMENT

Ana Paula Pérez Aguirre[1], Marina Rincon Torroella[1], Sunny Patel[1], Aryaman Shodhan[1], Kigongo Siriman[2], Manel Cole[1], Peter Waiswa[3], Catherine Maiteki-Sebuguzi[4], Neil Lobo[5], Soumyadipta Acharya[1]

[1]Johns Hopkins University, Center for Bioengineering Innovation & Design, Baltimore, MD, United States, [2]Vector Control Division, Kampala, Uganda, [3]Makerere University School of Public Health, Kampala, Uganda, [4]Ministry of Health, Kampala, Uganda, [5]University of Notre Dame, South Bend, IN, United States

Malaria control requires evaluating several epidemiological, entomological, and environmental factors to determine the most appropriate interventions to deploy in each region. With recent worldwide budget reductions, conducting a comprehensive analysis has become more important than ever to ensure effective use of resources. Through interactions with entomologists and decision-makers in Uganda we found that currently, vector control decisions are made after long periods of 6 months to 3 years, despite weekly data collection. The rate of data influx can surpass analytic capacity, leading to much of this data remaining unused and decisions being delayed. Stakeholders also mentioned that intervention decisions are centralized at the national level, with little input from district authorities. We propose VectorInsight, a dashboard intended to address these challenges by integrating entomological, epidemiological, and environmental data, analyzing trends and sending alerts, creating actionable insights, and supporting decentralized vector control. The dashboard also employs multimodal language models to analyze the data and assist local experts in identifying gaps in protection, using retrieval-augmented generation (RAG) to consult the Malaria Elimination Initiative's (MEI) Entomological Surveillance Planning Tool (ESPT). VectorInsight is directed towards district-level decision-makers to enable them to identify gaps in protection and address issues at the source. We have developed a preliminary prototype of the dashboard and are conducting thorough usability tests with experts from several countries' malaria control programs to evaluate the tool before deployment. Preliminary qualitative interviews suggest high user satisfaction, highlighting the potential of the tool to reduce malaria transmission without expensive interventions by improving district-level data analysis. VectorInsight thus shows potential to improve data usage in malaria-endemic countries, elevating the role of the districts in vector control by detecting and solving issues early.

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FORMATIVE ASSESSMENT OF ELECTRONIC MEDICAL RECORD READINESS FOR MALARIA SURVEILLANCE AHEAD OF A COUNTY-WIDE ROLLOUT IN SIAYA COUNTY WESTERN KENYA

Wycliffe O. Odongo[1], Kizito Omondi Obiet[2], Simon Kariuki[2], Victoria Seffren[1], Laura Steinhardt[1], Jimee Hwang[1], Samuel Owuor Akech[3], Titus Kwambai[3], Nelli Hrisceva Westercamp[1], Julie R. Gutman[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Kenya Medical Research Institute, Kisumu, Kenya, [3]Centers for Disease Control and Prevention, Kisumu, Kenya

Reliable facility-based malaria data are needed to monitor trends and evaluate intervention impact in western Kenya, yet electronic medical record (EMR) reporting remains fragmented across health facilities (HFs) and service delivery points (SDPs). In preparation for countywide rollout of the Taifa Care EMR, we conducted a formative assessment in four purposively selected primary and referral public HFs in Alego Usonga, Siaya County, to capture variation in service levels and operational contexts. We aimed to characterize EMR adoption, quantify the extent of digitization of malaria-related workflows, and identify gaps in training, data capture and linkage. Baseline EMR use was heterogeneous and largely concentrated in comprehensive care (HIV) clinics, with partial staff training in 3/4 HFs. Digitization varied by SDP: paper registers remained the primary source for malaria data in outpatient, antenatal care, laboratory, and pharmacy SDPs. All HFs concurrently used EMR and paper registers. Patient registration was digitized in 4/4 HFs, clinical notes and pharmacy in 3/4, and laboratory and billing/insurance in 2/4. Only 1/4 HF had structured malaria fields documented across laboratory, outpatient, and pharmacy workflows. No HF reported malaria laboratory results in the EMR; only 1/4 tracked antimalarial dispensing, and 1/4 had a local EMR backup. All HFs reported recurring internet and power interruptions. Findings highlight incomplete EMR coverage, weak linkages of diagnosis, testing, and treatment data, training and connectivity challenges, and reliance on parallel paper workflows as major barriers to EMR-supported malaria surveillance. The ongoing assessment and data validation will inform county-wide EMR rollout across 116 public HFs and provide critical data for targeted system strengthening to improve malaria burden monitoring and evaluation.

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STRUCTURE-BASED DESIGN OF A CPN60 NANOPARTICLE VACCINE FUSED WITH CSP PROTECTS MICE FROM MALARIA

Taha A. Ali, Thayne Henderson Dickey, Dashuang Shi, Wai Kwan Tang, Rui Ma, Richi Gupta, Palak N. Patel, Alaysies Queen, Myesha Singleton, Solomon Conteh, Patrick Emmet Duffy, Nichole Diane Salinas, Lynn E. Lambert, Niraj H. Tolia

National Institutes of Health, Bethesda, MD, United States

Nanoparticle platforms have transformed infectious disease vaccines through the periodic arrangement of antigens on their surface. Multivalent antigen presentation on nanoparticles increases the immune response to the antigen but also produces an adaptive immune response to the nanoparticle itself. Current malaria vaccines RTS,S/AS01 and R21/Matrix-M utilize nanoparticle platforms from an unrelated hepatitis B surface antigen to enhance immunogenicity. Nanoparticle platforms derived from malaria may improve upon these vaccines. Here, we identified *Plasmodium falciparum* Cpn60 (PfCpn60) as a nanoparticle platform for the CSP-based immunogen CSPj5c. PfCpn60 is an ATP-dependent chaperone protein and its orthologs have been shown to induce CD4+ and CD8+ T cell responses. PfCpn60 therefore has the potential to elicit a protective response, in addition to serving as a nanoparticle presentation platform for fused antigens. However, PfCpn60 transitions through multiple oligomeric states, reducing its stability as a nanoparticle presentation platform, and both the *N*- and *C*- termini of Cpn60 orient inward, preventing antigen attachment for surface display. To overcome these hurdles, we identified stability-enhancing mutations in PfCpn60 and engineered PfCpn60 with new *N*- and *C*-termini to allow for the attachment of antigens. The final construct PfCpn60CPHA assembles into a tetradecameric nanoparticle that can display as many as 28 subunits of CSPj5c in two different orientations. The fusion antigen CSP was shown to be accessible and strongly binds the CSP monoclonal antibodies CIS43 and mAb317. The CSP fusion nanoparticle formulated with AddaS03 adjuvant induced a strong CSP-specific antibody response and conferred sterile protection in mice when challenged with transgenic *P. berghei* sporozoites, demonstrating its viability as a potent malaria vaccine.

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A COMBINED DESIGNED CSP AND PFS48/45 INFECTION AND TRANSMISSION BLOCKING VACCINE FOR MALARIA

Niraj H. Tolia[1], Richi Gupta[1], Thayne Henderson Dickey[2], Nichole Diane Salinas[3], Palak N. Patel[4], Rui Ma[1], Dashuang Shi[1], Myesha Singleton[1], Tarik Ouahes[1], Thao Pham[1], Kazutoyo Miura[5], Carole A. Long[6], Lynn Lambert[1]

[1]National Institutes of Health, Bethesda, MD, United States, [2]NIH/NIAID/LMIV/HPISV, Bethesda, MD, United States, [3]NIAID, Silver Spring, MD,

United States, [4]National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States, [5]VRC/NIAID/NIH, Bethesda, MD, United States, [6]National Institutes of Health, Rockville, MD, United States

Malaria parasite life cycle complexity hinders vaccine development, making it challenging to design a vaccine that can effectively prevent infection and disease transmission. To address this challenge, we have developed a multistage vaccine that targets two independent stages of the life cycle: pre-erythrocytic infection and transmission. The self-assembled ferritin nanoparticle vaccine presents two immunogens, CSPj5c and 17-4, from the circumsporozoite and Pfs48/45 antigens, respectively. These immunogens were designed using structure-based, computational and human-guided approaches to retain protective epitopes and confer protection. The results show that immunization with CSPj5c-17-4-ferritin nanoparticles protects against sporozoite challenge in mice, demonstrating the vaccine's ability to prevent pre-erythrocytic infection. Furthermore, purified IgGs from immunized rabbits elicited potent transmission-reducing activity, indicating the vaccine's potential to block transmission. Notably, the addition of 17-4 to the vaccine improved immune responses to CSPj5c and protection from sporozoite challenge, suggesting a synergistic effect between the two antigens. The multistage malaria vaccine, CSPj5c-17-4-ferritin, holds promise for malaria control and represents a significant step towards developing a more effective single vaccine against this devastating disease.

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ASSESSMENT OF PROTECTIVE EFFICACY OF RECOMBINANT *PLASMODIUM VIVAX* CSP VACCINE CANDIDATES EXPRESSED IN HETEROLOGOUS SYSTEMS USING THE MURINE *P. BERGHEI* CHALLENGE MODEL

David L. Narum[1], Jonathan Renn[1], Kelly M. Rausch[1], Pinar Kemanli[1], Kayode Adeyemi[1], Nicholas J. MacDonald[1], Vu Nguyen[1], Sachy Orr-Gonzalez[1], Myesha Singleton[1], Raul Herrera[1], Fernando Cabezas Mejia[1], Karine Reiter[1], Beth B. Chen[1], Megan Grieco[2], Holly M. Torano[1], Lynn E. Lambert[1], Solomon Conteh[1], Emma K. Barnafo[1], Michael P. Fay[2], Justin Y.A. Doritchamou[1], Patrick E. Duffy[1]

[1]Laboratory of Malaria Immunology and Vaccinology, NIAID/NIH, Bethesda, MD, United States, [2]Biostatistics Research Branch, NIAID/NIH, Rockville, MD, United States

Plasmodium vivax malaria remains a significant global health challenge, causing millions of clinical cases annually. The *P. vivax* circumsporozoite protein (PvCSP) is orthologous to *P. falciparum* CSP, the active component in two WHO-approved anti-infection vaccines. One major challenge in PvCSP vaccine development is the presence of two major polymorphic alleles, VK210 and VK247, which typically feature 15-20 copies of 9-amino acid repeat sequences, contrasting with the single allele in *P. falciparum*. To advance a PvCSP-based vaccine, a panel of secreted recombinant PvCSP antigens was generated, including *Pichia pastoris*-secreted monomeric VK210 and VK247 proteins, produced in near full-length (G63) and shorter (E89) forms - located near the conserved KLKQP site. Additionally, a chimeric construct PvCSP-1047 was designed, containing 8 copies of both VK210 and VK247 repeats. A mammalian HEK293 system was utilized to produce monomeric chimeric forms with 4 or 16 repeats, as well as a mimic of WRAIR VMP001 (previously evaluated in a phase 1/2 trial). Using a novel filamentous fungus *Thermothelomyces heterothallica* (C1), which possesses a genome 4X larger than *P. pastoris*, we produced two secreted ferritin nanoparticles fused with PvCSP-1047, and a chemical conjugation platform was used to generate a PvCSP-1047-EPA conjugate. Each candidate underwent extensive biochemical and biophysical characterization to confirm purity, identity, and integrity. C57BL/6 mice were immunized on days 0, 21, and 42 using a water-in-oil emulsion, formulated at doses of 2 µg for monomers or 0.5 µg for conjugate/ferritin particles. Mice were challenged intradermally by 2000 salivary gland-purified *P. berghei* sporozoites expressing either VK210 or VK247 alleles. Vaccine candidates induced protection against homologous challenge (but not heterologous), with good correlation between ELISA titers and protection. The findings support utility of challenge studies for ranking candidate efficacy, and development continues for both the PvCSP-1047-EPA conjugate and ferritin nanoparticle, with planned assessments of human-use adjuvants.

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FUNCTIONAL VALIDATION OF T CELL EPITOPE IMMUNOGENICITY FOR NEXT-GENERATION MALARIA VACCINES

Alexander J. Laurenson, Franklin R. Toapanta, Kirsten E. Lyke, Matthew B. Laurens

University of Maryland School of Medicine, Baltimore, MD, United States

Developing an effective malaria vaccine is challenged by *Plasmodium falciparum* antigenic diversity and immune response heterogeneity across endemic populations. Existing vaccines offer partial, short-lived protection and are limited by strain-specific efficacy. Human Leukocyte Antigen (HLA) polymorphisms restrict immune recognition, further limiting vaccine effectiveness. To address these challenges, we previously developed a computational T cell Epitope Nomination (TEpiNom) tool integrating P. falciparum sequence diversity, T cell epitope-HLA binding affinity, and HLA allele frequency from endemic regions. This approach identified over 65,000 MHC I and 1,900 MHC II epitopes across multistage parasite antigens, down-selected for conservation and HLA-promiscuous coverage and prioritized for experimental validation. To inform whether top-ranked predicted epitopes translate into immunogenic vaccine candidates, we hypothesize that these epitopes will elicit robust, multifunctional CD8+ and CD4+ T cell responses in malaria-exposed individuals, with specific effector functions considered important for parasite control. Peripheral blood mononuclear cells from 20 HLA-typed malaria-exposed adults in Burkina Faso are being expanded and stimulated with peptide pools containing top-ranked epitopes spanning liver, blood, and sexual stage antigens. FluoroSpot assays will measure CD8+ (IFN-γ, Granzyme B, IL-2) and CD4+ (IFN-γ, TNF-α, IL-2) responses. Intracellular cytokine staining and flow cytometry with lineage, memory, and activation markers will assess T cell phenotypes and effector subset differentiation. We expect to identify epitopes consistently eliciting multifunctional CD8+ or CD4+ T cell responses across different HLA backgrounds. This will provide a prioritized immunogenic epitope panel to accelerate evidence-based design of optimized multivalent multistage *P. falciparum* vaccines with enhanced protective efficacy, broad HLA population coverage, and reduced immunogenic variability in endemic regions, while supporting TEpiNom as a rational vaccine design support framework.

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CHARACTERIZATION OF *PLASMODIUM VIVAX* CSP VACCINE CANDIDATES AS MONOMERIC PROTEINS, PROTEIN-PROTEIN CONJUGATES AND FERRITIN NANOPARTICLES

Karine Reiter[1], Raul Herrera[1], Vu Nguyen[1], Fernando Cabezas Mejia[1], Jianghai Zhu[2], Vinod Nair[2], Motoshi Suzuki[3], Chris Rowe[1], Kevin Cormier[3], Meghan Fitzwater[3], Duck-Yeon Lee[4], Nicholas J. MacDonald[1], Jacob Arras[5], Forrest Hoyt[2], Noelia Valbuena[6], Ronen Tchelet[6], Mark Emalfarb[6], Albert Jin[5], Patrick E. Duffy[1], David L. Narum[1]

[1]Laboratory of Malaria Immunology and Vaccinology, NIAID/NIH, Bethesda, MD, United States, [2]Electron Microscopy, Research Technologies Branch, NIAID/NIH, Bethesda, MD, United States, [3]Protein & Chemistry, Research Technologies Branch, NIAID/NIH, Bethesda, MD, United States, [4]Analytical Biochemistry Core, NHLBI/NIH, Bethesda, MD, United States, [5]National Institute for Biomedical Imaging and Bioengineering, NIH, Bethesda, MD, United States, [6]Dyadic Applied Biosolutions, Jupiter, FL, United States

Malaria remains a significant public health problem and caused 282 million cases globally in 2024 as reported by the WHO. RTS,S and R21 vaccines targeting circumsporozoite protein (CSP) provide clinical protection against *P. falciparum*, but no comparable *P. vivax* vaccine is available. In the Americas and Southeast Asia, most indigenous malaria cases are due to *Plasmodium vivax* (63% and 52%, respectively). Here, we report on

development of a panel of *P. vivax* CSP vaccine candidates produced in either *Pichia pastoris* as secreted monomeric proteins, or the novel fungal organism *Thermothelomyces heterothallica* (C1) as secreted self-assembled ferritin nanoparticles. *Pichia*-expressed proteins were near full-length individual alleles for VK210 and VK247, as well as a chimeric protein that included VK210 and VK247 repeats (PvCSP-1047). Identity of the purified recombinant proteins was confirmed by mass spectrometry and Edman sequencing. Biophysical characterization by multi-angle light scattering, dynamic light scattering (DLS) and atomic force microscopy (AFM) indicated these were prolate spheroid proteins. Secondary structures analyzed by far-UV circular dichroism spectroscopy showed a composition of about half random coil and half beta sheets and turns. To enhance immunogenicity, PvCSP-1047 was conjugated to a carrier protein ExoProtein A. Amino acid analysis determined the conjugate composition was 51.3% w/w PvCSP:EPA, or a 2.6:1 molar ratio, and light scattering showed an average hydrodynamic radius of 14.3 nm. We also recombinantly fused PvCSP-1047 protein to apoferritin from *Acidobacterium* at either amino or carboxyl ends, which generated self-assembled secreted nanoparticles during fermentation. Peptide mapping confirmed identity of purified PvCSP-ferritin. Light scattering, AFM and cryo-electron microscopy (2.23Å resolution) confirmed the presence of ferritin nanoparticles which formed decamers. This panel of characterized recombinant PvCSP proteins and nanoparticles is well-suited to assess vaccine efficacy in a murine challenge model.

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SAFETY AND IMMUNOGENICITY OF A MULTI-DOSE (10 DOSES) FORMULATION OF R21/MATRIX-M WITH THE PRESERVATIVE 2-PHENOXYETHANOL IN MALI: A CLUSTER-RANDOMIZED CONTROLLED TRIAL PROTOCOL

Mahamadou Dembele[1], Serge Ouoba[2], Toussaint Rouamba[3], Natama Magloire[4], Halidou Tinto[5], Kassoum Kayentao[6]

[1]Université des Sciences, Techniques et Technologies de Bamako (USTTB), Bamako, Mali, [2]Clinical Research Unit of Nanoro, Boulkiemdé, Burkina Faso, [3]Boulkiemdé, Burkina Faso, [4]Clinical Research Unit of Nanoro, Boulkiemdé, Burkina Faso, Burkina Faso, Burkina Faso, [5]IRSS, Ouagadougou, Burkina Faso, [6]Malaria Research & Training Center, Bamako 1805, Mali

Malaria causes more than 600,000 deaths each year worldwide, 90% of which are among children under five years of age living in sub-Saharan Africa.In 2023, the WHO recommended the R21/Matrix-M vaccine, which could prevent millions of malaria cases in children annually if high vaccination coverage is achieved.GAVI aims to immunise 52 million children by 2030, requiring $1.4 billion. To meet the demand and reduce costs, the Serum Institute of India is manufacturing the R21/MM vaccine with 2-Phenoxyethanol.The study aims to evaluate the safety and immunogenicity of the 10-dose formulation of R21/MM with the Preservative 2-Phenoxyethanol for future large-scale deployment of this formulation in malaria-endemic regions.In the control arm, children aged 5 to 36 months will receive a primary series of 3 doses of R21/MM (10 doses) in mass Expanded Programme on Immunisation (EPI) campaigns, 4 weeks apart before the start of the SMC campaign. The booster dose will be administered at 12 months, following national implementation guidelines.In the intervention arm, the 3-dose primary series will be administered monthly to all children in the SMC age group (3-59 months). The first three rounds of the SMC (July-August-September) will target the primary vaccination doses, while the fourth round will catch up with those who missed the previous doses. Children who haven't completed the three-dose primary series during the 4 SMC runs can receive their additional doses as part of routine EPI immunisation.The main outcome is the appearance of local and systemic reactogenicity signs and symptoms within 7 days after each vaccination (the day of vaccination and the next 6 days) for each study arm. Secondary outcomes include the levels of vaccine-induced anti-NANP IgG antibodies 28 days after the third dose (overall and by study arm). This trial aims to assess the safety and immunogenicity of the 10-dose (multiple dose) formulation of R21/Matrix-M with 2-Phenoxyethanol as a preservative, and to reduce malaria in children under 5 years. It will provide crucial evidence for national and global policies on malaria vaccine roll-out.

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GENETIC DIVERSITY, NATURAL SELECTION, AND IMMUNOLOGICAL FEATURES OF THE *PLAMODIUM VIVAX* CYRPA PROTEIN: IMPLICATIONS FOR VACCINE DEVELOPMENT

Carlos J. Castro-Cavadía[1], Diego Garzón-Ospina[2], Sindy P. Buitrago[3], Natalia Cepeda-Riaño[3], Maria F. Yasnot-Acosta[1]

[1]Universidad de Córdoba, Monteria, Colombia, [2]Universidad del Cauca, Popayán, Colombia, [3]Universidad Pedagógica y Tecnológica de Colombia, Tunja, Colombia

Malaria caused by *Plamodium vivax* remains a significant public health challenge, with vaccine development hindered by factors such as antigenic diversity and immune evasion. The Cysteine-Rich Protective Antigen (CyRPA), a key protein involved in erythrocyte invasion, has emerged as a promising vaccine candidate. How- ever, its genetic diversity and immunological properties have not been fully explored. This study aimed to analyze the genetic diversity, selective pressures, and antigenic potential of the pvcyrpa locus using 950 se- quences, including 42 newly obtained isolates from Colombia. The pvcyrpa gene displayed high nucleotide di- versity shaped by both natural selection and recombination. In-silico predictions identified B- and T-cell epitopes, encompassing mainly polymorphic regions, with strong binding affinities predicted for multiple HLA alleles. Notably, these epitopes overlapped with regions previously shown to elicit immune responses in natural in- fections, as reported in a recent study. Moreover, immune simulation of a multiepitope C-terminal construct predicted a robust humoral memory profile. Collectively, these genetic, epitope-mapping, and immune- simulation findings highlight the conserved C-terminal region of PvCyRPA as a strong, broadly reactive vaccine candidate, providing a rational basis for subsequent in-vitro and in-vivo validation.

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DIFFERENTIAL DYNAMICS OF NANP AND C-TERMINAL CSP IGG AVIDITY AND IGG SUBCLASS AFTER RTS,S/AS01$_E$ VACCINATION IN CHILDREN AND INFANTS FROM A PHASE 3 TRIAL

Lina Sánchez[1], Maria Lara Escandell[1], Dídac Macià[1], Chenjerai Jairoce[2], Maxmillian Mpina[3], Hermann Sorgho[4], Selidji Todagbe Agnandji[5], David Kwame Dosoo[6], Ben Gyan[6], Marta Vidal[1], Alfons Jiménez[1], Daniel Medina-Expósito[1], Robert Andrew Mitchell[1], Laura Puyol[1], Rebeca Santano[1], Diana Barrios[1], Sheetij Dutta[7], Simon K. Kariuki[8], Seth Owusu-Agyei[6], Kwaku Poku Asante[6], Halidou Tinto[4], Pedro C.P. Aide[2], Augusto Jose Nhabomba[2], Benjamin Mordmüller[9], Claudia Daubenberger[10], John Waitumbi[11], Ruth Aguilar[1], Gemma Moncunill[1], Carlota Dobano[1]

[1]Barcelona Institute for Global Health (ISGlobal), Barcelona, Spain, [2]Manhiça Health Research Center (CISM), Maputo, Mozambique, [3]Biomedical Research and Clinical Trials Department, Ifakara Health Institute, Ifakara, United Republic of Tanzania, [4]Unité de Recherche Clinique de Nanoro, Institut de Recherche en Sciences de la Santé, Nanoro, Burkina Faso, [5]CERMEL, Lambarene, Gabon, [6]Kintampo Health Research Centre, Kintampo, Ghana, [7]WRAIR, Silver Spring, MD, United States, [8]KEMRI-CDC, Kisumu, Kenya, [9]Department of Medical Microbiology, Radboud University Medical Center, Nijmegen, Netherlands, [10]Swiss Tropical and Public Hlth Inst, Basel, Switzerland, [11]US Army Medical Research Directorate-Africa, Kenya Medical Research Institute, Nairobi, Kenya

The RTS,S/AS01$_E$ (RTS,S) malaria vaccine confers partial and time-limited protection against *Plasmodium falciparum*, with correlates of protection primarily focusing on peak IgG responses to NANP repeats of the circumsporozoite protein (CSP). The role of antibody quality, epitope specificity, and subclass distribution remains incompletely defined. In this longitudinal study nested within a phase 3 trial (NCT00866619), we

quantified IgG avidity (n=1136) and subclass responses (n=2232) to CSP Full Length, the CSP NANP repeats and the C-terminal (Cterm), using multiplex assays. IgG avidity was measured using urea as chaotropic agent. Measurements were taken at five timepoints before and after primary and booster vaccination in infants and children from seven African sites. Cterm avidity remained stable after primary vaccination, but NANP avidity declined during the IgG maintenance phase (p=0.001). Booster vaccination increased Cterm avidity (p=0.0001) but did not enhance NANP avidity beyond primary levels (p=0.619). In infants, higher baseline NANP avidity, likely reflecting maternal antibodies, declined after vaccination, but this was not observed for Cterm responses. IgG subclass analyses showed marked domain- and age-dependent differences. IgG1 response increased after primary vaccination for C-term and NANP, whereas boosting preferentially enhanced Cterm IgG1 responses (FC: 1.5 [95% CI 1.4 - 1.7]). Infants showed higher IgG2 responses than children for both NANP and Cterm, with IgG2 further increasing after boosting. IgG3 peaked after primary vaccination and declined thereafter with a limited increase after the booster. IgG4 responses emerged during the maintenance phase and were strongly boosted for Cterm (FC: 5.8 [95% CI 4.9 - 6.8]) but remained minimal for NANP (FC: 1.3 [95%CI 1.2 -1.5]). The stronger induction and longer maintenance of IgG avidity to Cterm, together with the different dynamics in subclass switching, suggest differences in antibody responses to specific CSP regions. Ongoing analyses will assess associations with malaria protection to refine correlates of protection and inform vaccine optimization.

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PRODUCTION OF RECOMBINANT *PLASMODIUM VIVAX* CIRCUMSPOROZOITE PROTEIN VACCINE CANDIDATES IN YEAST AND FILAMENTOUS FUNGI

Fernando Jose Cabezas Mejia[1], Vu Nguyen[1], Nicholas J. MacDonald[1], Karine Reiter[1], Raul Herrera[1], Noelia Valbuena[2], Ronen Tchelet[2], Mark Emalfarb[2], Patrick Emmet Duffy[1], David L. Narum[1]

[1]Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States, [2]Dyadic Applied Biosolutions, Jupiter, FL, United States

Plasmodium vivax remains the most geographically widespread cause of malaria, with cases surging from 3.8 million in 2020 to 9.9 million in 2024. Because mortality data often goes unrecorded, the parasite's true global impact remains hidden. To meet the urgent need for new interventions, our research focuses on developing a *P. vivax* (Pv) vaccine candidate targeting the circumsporozoite protein (CSP). Initially, we produced full-length recombinant PvCSP (VK210 and VK247 strains) using the yeast *Pichia pastoris*. After identifying two cleavage sites at G63 and E89 in the amino terminus, we engineered new cell lines of both alleles and a new chimeric construct, PvCSP-1047, which fuses eight copies of both the VK210 and VK247 repeats. In 5L bioreactor fermentations, these yeast strains achieved yields between 0.54 g/L and 0.79 g/L in fermentation supernatant. Proteins were purified via traditional column chromatography. In parallel, we explored the filamentous fungus *Thermothelomyces heterothallica* (C1) as a high-yield, low-cost expression system. Using glucose as a constitutive inducer, C1 successfully secreted self-assembling ferritin nanoparticles fused with the PvCSP-1047 chimera. The N-terminal fusion (PvCSP-1047-FN) reached a yield of 6.7 ± 1.6 g/L fermentation supernatant in just 72 hours of total fermentation time. The ferritin nanoparticles were purified using C-tag affinity chromatography. The panel of purified recombinant PvCSP proteins and nanoparticles were evaluated by Coomassie blue stained SDS-PAGE gels, Western blot, and analytical size exclusion chromatography. Collectively, these results demonstrate highly productive and scalable platforms for pilot-scale vaccine manufacturing.

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HIGH LEVELS OF ANTI-PFEMP1 ANTIBODIES ARE ASSOCIATED WITH LOW LEVELS OF PROTECTION FROM *PLASMODIUM FALCIPARUM* MOSQUITO BITE CHMI AFTER PFSPZ VACCINATION

Brian A. Joughin[1], Wonyeong Jung[2], Sarper Okuyan[3], Hyacintha Bisimwa[4], Yonatan Zur[3], Daphne A. Diloretto[4], Mamie Li[5], Robert Seder[6], B. Kim Lee Sim[7], Stephen L. Hoffman[8], Facundo Batista[3], Michael Mina[9], Marion Avril[10], Douglas Lauffenburger[1], Stephen J. Elledge[5], Galit Alter[11], **Jonathan D. Herman**[4]

[1]MIT, Cambridge, MA, United States, [2]Univeristy of Korea, Seoul, Republic of Korea, [3]Ragon Institute, Cambridge, MA, United States, [4]UCLA, Los Angeles, CA, United States, [5]Brigham and Women's Hospital, Boston, MA, United States, [6]NIAID/NIH, Bethesda, MD, United States, [7]Sanaria Inc., Rockville, MD, United States, [8]Sanaria Inc, Rockville, MD, United States, [9]Harvard School of Public Health / Harvard Medical School, Boston, MA, United States, [10]MalarVx, Inc, Seattle, WA, United States, [11]Ragon Institute of MGH, MIT, and Harvard, Cambridge, MA, United States

Highly protective long-lasting malaria vaccines are needed for elimination and eradication of malaria. Although vaccines based on attenuated *Plasmodium falciparum* sporozoites (PfSPZ) can be highly protective [BJ1] against controlled human malaria infection (CHMI), in most trials a minority of vaccinees are not protected for reasons that are still unclear. To address this gap, we developed a *P. falciparum* phage display immunoprecipitation sequencing (PhiP-Seq) assay that enabled us to profile the humoral antibody response to overlapping 56-amino acid peptides tiling the entire pre-erythrocytic malaria proteome in a clinical trial of the radiation-attenuated Sanaria® PfSPZ vaccine in a malaria-naive population that underwent mosquito-bite CHMI challenge. Using this unbiased approach, we found that antibodies targeting PfEMP1 var gene encoded proteins, traditionally thought to be specific to the erythrocytic life cycle stage, were enriched in the 10 non-protected individuals relative to 31 protected individuals, suggesting a novel immune evasion mechanism. Further, mathematical models that predicted patient protection status after vaccination largely rely on a low anti-PfEMP1 antibody response. Altogether, this highlights anti-PfEMP1 antibodies as potential biomarkers of non-protection and suggests a novel mechanism of immune evasion that may inform the rational design of next-generation malaria vaccines.

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DISCOVERY OF PROTECTIVE ANTIBODIES THAT TARGET THE NOVEL PYROGLUTAMYLATED *PLASMODIUM FALCIPARUM* CIRCUMSPOROZOITE PROTEIN EPITOPE (PGLU-CSP)

Cherrelle Dacon[1], Re'em Moskovitz[2], Kristian Swearingen[3], Lais Da Silva Pereira[4], Yevel Flores-Garcia[5], Maya Aleshnick[6], Sachie Kanatani[5], Barbara Flynn[7], Alvaro Molina-Cruz[8], Kurt Wollenberg[9], Maria Traver[10], Payton Kirtley[6], Lauren Purser[1], Marlon Dillon[7], Brian Bonilla[7], Adriano Franco Casin[1], Hasmik Manukyan[1], Samantha Petros[1], Jake Kritzberg[1], William Zhong[1], Gonzalo Gonzalez Paez[2], Priya Gupta[11], Melanie Shears[12], Joseph Pazzi[13], Joshua Edgar[13], Andy Teng[13], Arnel Belmonte[14], Kyosuke Oda[14], Safiatou Niare Doumbo[15], Ludmila Krymskaya[10], Jeff Skinner[16], Shanping Li[16], Suman Ghosal[9], Kazutoyo Miura[7], Daria Nikolaeva[7], Kassoum Kayentao[15], Aissata Ongoiba[15], Ashley Vaughan[11], Joseph Campo[13], Carolina Barillas-Mury[8], Wathsala Wijayalath[14], Azza Idris[7], Peter Crompton[16], Photini Sinnis[5], Brandon Wilder[6], Fidel Zavala[5], Robert Seder[7], Ian A. Wilson[2], Joshua Tan[1]

[1]Antibody Biology Unit, National Institutes of Health, Rockville, MD, United States, [2]The Scripps Research Institute, San Diego, CA, United States, [3]Institute for Systems Biology, Seattle, WA, United States, [4]National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States, [5]Johns Hopkins University, Baltimore, MD, United States, [6]Oregon Health and Science University, Beaverton, OR, United States, [7]Vaccine Research Center, National Institutes of Health, Bethesda, MD, United States, [8]Laboratory of Malaria and Vector Research, National Institutes of Health, Rockville, MD, United States, [9]Bioinformatics and Computational

Bioscience Branch, National Institutes of Health, Bethesda, MD, United States, [10]Laboratory of Immunogenetics, National Institutes of Health, Rockville, MD, United States, [11]Seattle Children's Research Institute, Seattle, WA, United States, [12]University of Washington, Seattle, WA, United States, [13]Antigen Discovery Incorporated, Irvine, CA, United States, [14]Naval Medical Research Command, Silver Spring, MD, United States, [15]University of Sciences, Technique and Technology of Bamako, Bamako, Mali, [16]Malaria Infection Biology and Immunity Section, National Institutes of Health, Rockville, MD, United States

The most clinically advanced malaria vaccines and monoclonal antibodies (mAbs) target the *Plasmodium falciparum* circumsporozoite protein (PfCSP) which coats the sporozoite surface. However, the diversity of sporozoite surface antigens remains largely uncharacterized. Thus, we developed an antigen agnostic workflow for screening B cells for reactivity towards whole sporozoites and recombinant PfCSP in tandem. Using this approach, we isolated 10 mAbs that bound to sporozoites, but not to any form of recombinant PfCSP tested. The most potent mAb (MAD21-101) reduced liver parasite load by more than 90% in an *in vivo* humanized-liver model of infection. Surprisingly, subsequent proteomic and structural analyses revealed that the mAbs recognize a form of PfCSP that has undergone two sequential parasite-mediated, post-translational modifications at the N-terminus: (1) proteolytic cleavage and (2) subsequent pyroglutamylation to generate an N-terminal pyroglutamate (pGlu) residue. The epitope of these 'pGlu-CSP' mAbs is absent from WHO-approved vaccines and is different from the epitopes of previously isolated anti-PfCSP mAbs. Therefore, pGlu-CSP mAbs are unlikely to interfere with currently deployed vaccines and have a unique pathway to development. pGlu-CSP mAbs are incredibly rare. Thus, we have recently expanded our isolation strategy to include both IgG and IgM antibodies to increase the throughput of pGlu-CSP mAb discovery. Flow cytometry analyses of plasma from 941 *P. falciparum*-exposed donors has identified donors of interest who retain polyclonal reactivity to whole sporozoites after adsorption of PfCSP-specific and pGlu-CSP-specific antibodies. Our current work is focused on characterizing new pGlu-CSP mAbs that were isolated from these down-selected donors using both antigen-agnostic Beacon assays and pGlu-CSP probe sorting. In parallel, ongoing work is focused on investigating the utility of pGlu-CSP mAbs in multi-specific strategies, assessing the relative potency of MAD21-101 versus best-in-class PfCSP mAbs and improving MAD21-101 potency using *in vitro* mutagenesis approaches.

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LONGITUDINAL ANALYSIS OF *PLASMODIUM FALCIPARUM* CSP AND AMA1 ANTIGENIC DIVERSITY IN HUMAN AND MOSQUITO INFECTIONS IN WESTERN KENYA REVEALS HIGH DIVERSITY AND RAPID TEMPORAL FLUCTUATIONS WITH LIMITED REPRESENTATION OF VACCINE VARIANTS

Evalyne N. Wambugu[1], Francis M. Ndungu[1], Steve M. Taylor[2], Christine F. Malkwalter[2], Wendy P. O`Meara[2]

[1]KEMRI-Wellcome Trust Research Programme, Kilifi, Kenya, [2]Duke University, Durham, NC, United States

The WHO-recommended malaria vaccines RTS,S/AS01 and R21/Matrix-M target *Plasmodium falciparum* circumsporozoite protein (*Pf*CSP) and are used in African children, but their effectiveness is limited by extensive antigenic diversity among circulating parasites. Most current and candidate vaccines, including those targeting *Pf*CSP and apical membrane antigen 1 (*Pf*AMA1), are based on a single antigen variant, which limits protection against diverse parasite variants. This study aimed to characterize the antigenic diversity of *Pf*CSP and *Pf*AMA1, examine temporal changes, and assess the extent of mismatch with the currently approved and candidate malaria vaccine variants. We conducted a longitudinal antigenic analysis of the *Pf*csp C-terminal and *Pf*ama1 domain 1 sequences obtained from symptomatic and asymptomatic human (n=2736) and mosquito infections (n=371) collected in Webuye, Kenya, between 2017 and 2021. Antigenic diversity was tracked over time, and amino acid sequences were compared to the 3D7 vaccine variant used in RTS,S/AS01, R21/Matrix-M, and AMA1-based vaccine candidates. A total of 296 *Pf*csp and 353 *Pf*ama1 haplotypes were identified. Temporal analyses revealed distinct patterns of persistence: a subset of haplotypes persisted for long periods (>30 months), over half (62.5% of *Pf*csp and 62.88% of *Pf*ama1) were detected in only a single month, and others exhibited episodic circulation. Haplotypes detected in both humans and mosquitoes showed the longest persistence, with median durations of 21 months (95% CI: 14–26) for *Pf*csp and 13.5 months (95% CI: 11–18) for *Pf*ama1, whereas human-only haplotypes were short-lived with median persistence of 1 month (95% CI: 1–1) for both antigens. Only 6.1% of *Pf*csp sequences and 5.6% of *Pf*ama1 sequences matched the respective 3D7 vaccine variants. Epitope analysis showed high polymorphism in *Pf*csp DV10, Th2R, Th3R, and *Pf*ama1 C1L regions relative to the 3D7 variant. These results highlight the urgent need for next-generation malaria vaccines that account for real-world diversity, elicit broad, variant-transcending immunity, and are informed by continuous molecular surveillance.

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REVOLUTIONIZING MALARIA RESEARCH, ESPECIALLY IN AFRICA, THROUGH CONTROLLED HUMAN MALARIA INFECTION (CHMI) BY INJECTION OF ASEPTIC, PURIFIED, CRYOPRESERVED *PLASMODIUM FALCIPARUM* POROZOITES CALLED PFSPZ CHALLENGE

Thomas L. Richie[1], Stephen L. Hoffman[2]

[1]Sanaria Inc., Rockville, MD, United States, [2]Sanaria Inc, Rockville, MD, United States

In 2026 it was reported that when aseptic, purified, cryopreserved *Plasmodium falciparum* (Pf) sporozoites (SPZ) (PfSPZ Challenge) were administered by intradermal (ID) injection to Kenyan adults immunized with R21-MatrixM, there was 75% sterile protection against Pf infection. When the PfSPZ were administered by direct venous inoculation (DVI) there was 0% protection. This proved for the first time that R21 protected by inducing anti-PfCSP antibodies neutralizing PfSPZ in the skin and did not eliminate Pf-infected hepatocytes. In contrast, immunization with attenuated PfSPZ had 100% protection against CHMI by DVI in Tanzanian and Malian adults, implicating cellular immunity in the liver as the protective mechanism. There was no mosquito bite CHMI at the Kenyan research site; had there been, this definitive result could not have been obtained using mosquito bite CHMI, which combines intradermal with intracapillary sporozoite deposition. Since the first trial in Nijmegen in 2010, PfSPZ Challenge has been used safely for CHMI in 1648 individuals in 6 countries in Africa, 5 in Europe, and at multiple US sites. It has been used to assess pre-erythrocytic, asexual erythrocytic and transmission blocking vaccines, drugs, the impact of naturally acquired and vaccine-induced immunity and sickle trait on susceptibility to infection and to establish that the Dantu blood group provides high-level protection against early, non-clinical, Pf infections. In Kenya in a period of 4 years 12 Kenyans received their PhDs based on studies done with PfSPZ Challenge. A major regulatory agency has indicated that Phase 3 clinical trials assessing protection against CHMI with 3 different strains of Pf from different geographic locations instead of field trials can be used to support licensure, thereby dramatically reducing costs of Phase 3. Results to date and a plan for expanding the use of CHMI in the future will be discussed.

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IMMUNOGENICITY AND FUNCTIONAL ACTIVITY OF MRNA-LNP COMBINATIONS ENCODING PFCSP, PFS230D1M AND PFS25

Clifford T H Hayashi[1], Yi Cao[1], Tucker Harju[1], Abhai K. Tripathi[2], Fidel P. Zavala[3], Garima Dwivedi[4], Drew Weissman[4], Nirbhay Kumar[1]

[1]George Washington University, School of Public Health, Washington, DC, United States, [2]Johns Hopkins Malaria Research Institute, Baltimore, MD, United States, [3]Johns Hopkins University School of Public Health, Baltimore, MD, United States, [4]University of Pennsylvania, Philadelphia, PA, United States

Targeting multiple stages of the *P. falciparum* parasite is a next step in vaccine development for the pursuit of malaria elimination. mRNA-LNP

vaccine technology is well suited for the development of combination vaccines as multiple antigens can be combined with ease. We evaluated combinations of mRNA-LNPs encoding the pre-erythrocytic antigen, PfCSP, and transmission blocking targets (Pfs230D1M or Pfs25). A dose response study identified the optimal vaccine dose of PfCSP-mRNA (10µg vs 5µg) using BALB/c, C57BL/6 (inbred) and CFW (outbred) strains of mice (N=10 per group), immunized in a 3-immunization regimen given at 4-week intervals. Immunogenicity was measured by anti-PfCSP endpoint ELISAs, and the protective efficacy was evaluated by challenging mice with transgenic *P. berghei* expressing PfCSP and luciferase (PbPfCSPLuc) and measuring the liver-stage burden by quantifying bioluminescence using IVIS. All groups exhibited high anti-PfCSP titers, with mean titers ranging from 1.6×10^6 to 6.9×10^6, and greater than 80% liver-stage burden reduction. There was no significant difference between the 5µg and 10µg doses, regardless of mouse strain. However, CFW mice had lower antibody titers and liver-stage burden reduction than the inbred mouse strains. IgG subclass ratios indicated that C57BL/6 mice were Th1 skewed, BALB/c mice showed balanced to Th2 responses, and CFW mice were strongly Th2 skewed. There was no difference in anti-PfCSP avidity between groups. Next, we immunized CFW mice (N=10 per group) with 5µg PfCSP-mRNA in combination with 1µg Pfs25-mRNA or 1µg Pfs230D1M-mRNA. Single antigen immunized groups were used for comparison. All PfCSP-mRNA immunized groups showed greater than 80% liver stage burden reduction with no difference between the mRNA combinations and PfCSP-mRNA alone. Transmission blocking activity is being evaluated using SMFA. Our studies demonstrate that a combination of PfCSP-mRNA with either Pfs25-mRNA or Pfs230D1M-mRNA does not compromise protective efficacy. These studies also provide a rationale for further evaluation of combination vaccines in non-human primates and future clinical trials.

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IMMUNOGENICITY AND IMMUNE PROFILING OF AN ENGINEERED CSP SUBDOMAIN-BASED NANOPARTICLE VACCINE AGAINST *PLASMODIUM VIVAX*

Pooya Mahdavi

University of South Florida, Tampa, FL, United States

Malaria caused by *Plasmodium vivax* remains a major global health challenge due to its relapsing liver-stage infection. Targeting pre-erythrocytic stages is a key strategy for vaccine development. The dominant circumsporozoite protein (CSP) surface antigen, which is used in the current *P. falciparum* vaccines, RTS,S, and R21/Matrix-M, remains a leading candidate for next-generation vaccine design. Here, we identified a B-cell epitope of CSP via epitope mapping and engineered a CSP subdomain-based nanoparticle vaccine and evaluated its immunogenicity and immune profile following immunization. 6-8 weeks old BALB/c mice were immunized subcutaneously with GLA-SE-adjuvanted formulations, and antigen-specific responses were assessed 42 days post-immunization. Immunized mice developed robust antigen-specific IgG responses with high titers across all vaccine groups. IgG subclass analysis demonstrated a predominantly IgG1-biased response with detectable IgG2a and IgG2b, indicating a strong humoral response with a mixed subclass distribution. Differences in antibody magnitude across formulations suggest that construct design influences immunogenicity. Functional activity of vaccine-induced antibodies is being evaluated further by using inhibition of liver stage development assays. Ongoing studies characterize the immune response by flow cytometric analysis of splenocytes and spatial transcriptomics of draining lymph nodes, providing insights into the cellular and spatial organization of vaccine-induced immunity. These findings support the development of subunit protein CSP-based nanoparticle vaccine and establish a framework for linking immunogenicity, functional activity, and immune architecture in *P. vivax* vaccine design.

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SERUM ANTI-PFCSP ANTIBODY PROFILES IN INFANTS PROTECTED BY PFSPZ VACCINE ADMINISTERED TO MOTHERS BEFORE CONCEPTION

Jonathan P. Renn[1], Matthew V. Cowles[1], Halimatou Diawara[2], Sara A. Healy[1], Justin Y.A. Doritchamou[1], Natasha KC[3], Robert D. Morrison[1], B. Kim Lee Sim[4], Thomas Laurence Richie[4], Alassane Dicko[2], Stephen L. Hoffman[3], Patrick Emmet Duffy[1]

[1]Laboratory of Malaria Immunology and Vaccinology, NIAID/NIH, Bethesda, MD, United States, [2]Malaria Research and Training Center, University of Sciences, Techniques and Technologies, Bamako, Mali, [3]Sanaria Inc, Rockville, MD, United States, [4]Sanaria Inc., Rockville, MD, United States

Malaria is a leading cause of infant morbidity and mortality in endemic regions. We recently published that *Plasmodium falciparum* sporozoite (PfSPZ) Vaccine is safe and prevents parasitemia and clinical malaria during pregnancy in women who conceive within 21 months of last vaccine dose. Surprisingly, we also found that preconception PfSPZ Vaccine reduces malaria risk during infancy in their offspring. Here, we developed a multiplex platform to dissect maternal and infant antibody responses to PfCSP fragments and variants that might contribute to protection during infancy. Our multiplex panel contains full-length PfCSP NF54 and different domains derived from the NF54 sequence including the NANP major repeat peptide, the NVDP minor repeat peptide that binds the highly functional monoclonal antibody L9, the junctional region that binds the highly functional monoclonal antibody CIS43, and the C-terminal Tsr domain. Additionally, we included 6 variant Tsr domains. A vaccine sieving effect has been observed in children who received RTS,S/AS01 and these sites of sieving were observed in the Th2R and Th3R epitopes in the Tsr domain (N Engl J Med. 2015 Oct 21;373(21):2025-2037). We produced our 6 recombinant Tsr domains based on PfCSP variants from Malian isolates collected during a prior PfSPZ Vaccine trial in Malian adults, wherein mutations in either the Th2R, Th3R or both epitopes were observed to be elevated in the vaccine group or the placebo group. Pilot experiments with adults immunized with PfSPZ Vaccine show excellent reactivity to our panel of antigens suggesting our multiplex serological approach is valid. We will report antibody profiles at delivery and 6 and 12 months of infant age, and their associations to protection and vaccine efficacy.

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OPTIMIZING *IN VITRO* PRODUCTION OF LATE ARRESTING REPLICATION COMPETENT GENETICALLY ATTENUATED *PLASMODIUM FALCIPARUM* SPOROZOITES CALLED PFSPZ-LARC2

Minglin Li[1], Tint Tint Wai[2], Lixin Gao[2], Asha Patil[1], Yonas Abebe[1], Kajol Pradhan[1], Marlon Johnson[1], Natasha KC[3], Ehud Inbar[4], Tales V. Pascini[1], Bethany Jenkins[5], B. Kim Lee Sim[1], Stephen L. Hoffman[3]

[1]Sanaria Inc., Rockville, MD, United States, [2]Sanaria, Rockville, MD, United States, [3]Sanaria Inc, Rockville, MD, United States, [4]SANARIA, Rockville, MD, United States, [5]Sanaria, Inc., Rockville, MD, United States

Mosquito-derived *Plasmodium falciparum* (Pf) sporozoites (SPZ) (mPfSPZ) are the active component of Sanaria® PfSPZ vaccines. However, large-scale malaria elimination efforts require a scalable *in vitro* production platform. We have reported that infectious PfSPZ of the NF54 strain of Pf (iPfSPZ) can be generated *in vitro*, but we have not yet achieved consistent production of iPfSPZ of the quality of mPfSPZ. We have advanced from early arresting replication deficient radiation attenuated PfSPZ called PfSPZ Vaccine to late arresting replication competent genetically attenuated PfSPZ called PfSPZ-LARC2 Vaccine because of expected enhanced potency and decrease cost of vaccine doses. Building on prior work, we optimized culture conditions using a feeder cell system (MSQ43) embedded in alginate gel, which enhances oocyst formation, oocyst size, parasite amplification and PfCSP expression. We evaluated the impact of pH changes in the media used at the exflagellation, ookinete, and oocyst stages of development. Both exflagellation and ookinete media exhibited

pH instability, necessitating storage at –20 °C, and use within 4-6 weeks for exflagellation medium and storage at 4 °C, and use within 3 weeks for ookinete medium. In contrast, the oocyst medium remained stable at 4 °C, with the optimal pH being more acidic. Under optimized conditions, PfLARC2 achieved ~1.5% conversion from stage V gametocytes to 8 day-oocysts, representing a 3-6-fold improvement over *in vivo* rates. LARC2 oocysts reached diameters of ~30 µm, larger than those produced with wild type parasites at the same time point, but still smaller than their *in vivo* counterparts. Ongoing studies are focused on alteration of nutritional and environmental factors to further enhance maturation and yield. These findings support the feasibility of scalable *in vitro* production of high-quality sporozoites (iPfSPZ), which will be required to the universal immunization that will be required for geographically focused malaria elimination campaigns.

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DEVELOPMENT OF A NOVEL MULTIVALENT MALARIA VACCINE ANTIGEN UTILIZING CIRCULAR MIMOTOPE PEPTIDES TO TARGET MULTIPLE STAGES OF *PLASMODIUM* LIVER INFECTION.

Sung-Jae Cha, Sunny Kim, Olga Uchakina, Manas Paresh Patel
Mercer University, Macon, GA, United States

Previously, using a phage display library, we identified circular mimotope peptides that structurally replicate the conformation of *Plasmodium* sporozoite ligands essential for liver infection. These 12-amino-acid circular peptides mimic the conformational epitopes of parasite ligands that mediate critical host-cell interactions. Fine epitope mapping, utilizing antibodies raised against these peptides, identified specific parasite epitopes responsible for Kupffer cell traversal and hepatocyte infection. Notably, these small peptides elicit potent protective immunity in murine models by inducing neutralizing antibodies with stronger efficacy compared to those raised against native parasite epitope sequences. Leveraging their minimal size and structural stability, we integrated these circular peptides into a single multivalent fusion antigen designed to disrupt three discrete stages of sporozoite liver infection: sinusoidal attachment, Kupffer cell traversal, and hepatocyte invasion. Specifically, we engineered a trivalent construct fusing the PfCSP (NANP)5 repeat peptide with the P39 peptide (Kupffer cell traversal) and the HP1 peptide (hepatocyte invasion). In a murine model, this trivalent fusion antigen elicited significantly higher immunogenicity and protective efficacy compared to single- or double-epitope controls. Our findings demonstrate that a multi-epitope strategy utilizing conformationally stable circular peptides provides broad-spectrum protective immunity. This approach represents a promising platform for enhancing malaria control and mitigating the emergence of vaccine-resistant parasite strains through a single antigen.

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PFCSP MINOR-REPEAT ARCHITECTURE DIFFERENTIALLY REGULATES EPITOPE-SPECIFIC MONOCLONAL ANTIBODY PROTECTION

Ganchimeg Bayarsaikhan[1], Yevel Flores-Garcia[2], Prabhanshu Tripathi[3], Jennifer Suurbaar[4], Azza Idris[5]
[1]Ragon Institute of MGB, MIT and Harvard, Cambridge, MA, United States, [2]Johns Hopkins Univ, Baltimore, MD, United States, [3]Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States, [4]UNIVERSITY OF GHANA, Tamae, AA, Ghana, [5]NIH Vaccine Res Center NIAID, Cambridge, MA, United States

Monoclonal antibodies targeting the *Plamodium falciparum* circumsporozoite protein (PfCSP) are emerging as promising tools for malaria prevention, yet the extent to which natural parasite diversity affects their efficacy remains a critical question for real-world deployment. The PfCSP repeat region exhibits global variation in the number and arrangement of NANP major and NVDP minor repeat motifs, which form key antibody epitopes. To address this, we combined large-scale PfCSP sequence analysis with a transgenic *Plasmodium berghei* platform expressing defined and field-derived PfCSP repeat architectures. We evaluated clinically relevant monoclonal antibodies targeting distinct epitopes, including the minor-repeat–specific L9LS, the junctional antibody CIS43LS, and the major-repeat antibody 224LS. We find that protection by L9LS is dependent on minor-repeat valency: parasites containing two or more NVDP motifs remain highly susceptible, whereas a single NVDP motif significantly reduces protection. In contrast, CIS43LS and 224LS maintain robust efficacy across all repeat configurations tested. Importantly, parasites expressing PfCSP sequences from Ghana and Kenya field isolates remain sensitive to L9LS despite differences in repeat spacing and organization. These results demonstrate that leading anti-PfCSP antibodies are largely resilient to naturally circulating parasite diversity and identify minimal epitope requirements for protection. This work provides a framework for predicting field efficacy and informs the design of next-generation monoclonal antibodies and vaccines for malaria prevention.

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GROWTH EFFECTS OF *GIARDIA LAMBLIA* AND *CRYPTOSPORIDIUM SPP*. IN EARLY CHILDHOOD: A SYSTEMATIC REVIEW

Ashley Wade[1], Lester Ivan Gutiérrez[2], Anna Seaman[3], Zehra Jamil[4], Divya Mehta[5], Banks Kooken[6], Sarah Wright[7], Luther Bartelt[5]
[1]Icahn School of Medicine at Mount Sinai, New York, NY, United States, [2]Universidad de Costa Rica, San José, Costa Rica, [3]UNC Gillings School of Global Public Health, Chapel Hill, NC, United States, [4]Aga Khan University, Biological & Biomedical Sciences, Karachi, Pakistan, [5]UNC School of Medicine, Chapel Hill, NC, United States, [6]Indiana University School of Medicine, Indianapolis, IN, United States, [7]University of North Carolina at Chapel Hill, Health Sciences Library, Chapel Hill, NC, United States

Giardia lamblia and *Cryptosporidium spp*. are associated with childhood growth faltering, but reported effects vary widely. We conducted a systematic review examining the effect of these pathogens on longitudinal growth in children ≤ 5 years, considering symptomatic status, study design, geography, and other variables. Seven databases were searched through April 2025 for studies of children ≤ 5 years with *Giardia* or *Cryptosporidium* detection, a comparison group, and anthropometry at ≥2 timepoints. Outcomes included height-for-age and weight-for-age z-scores. Of 7,558 non-duplicate articles, 809 underwent full-text review and 32 met extraction criteria. Studies were categorized by statistical confidence: high-confidence (multivariate analysis with significant association in ≥1 subgroup) or low-confidence (univariate analysis, nominally significant, or borderline results) for negative or null growth associations. Of 20 *Giardia* studies examining HAZ, 11 (55%) found deficits, with 9 classified as high-confidence. Of 18 *Cryptosporidium* studies, 14 (78%) found HAZ deficits, with 7 classified as high-confidence. For *Giardia*, HAZ deficits were found in 5/6 (83%) asymptomatic-only and 6/14 (43%) mixed-status studies. For *Cryptosporidium*, HAZ deficits were found in 5/5 (100%) symptomatic-only, 7/9 (78%) mixed-status, and 2/4 (50%) asymptomatic-only studies. Birth cohorts detected deficits more often than cohorts enrolling post-infancy (73-89% vs 25-29% across pathogens). Significant heterogeneity was observed in exposure definitions, comparison groups, pathogen detection timing, and follow-up duration. The majority of studies found negative growth associations for both pathogens, many of high statistical confidence. The differing patterns by symptomatic status suggest distinct growth impact pathways, with implications for pathogen-specific interventions. The greater birth cohort deficits may support the early-life window hypothesis for growth effects. Significant heterogeneity underscores the need for standardized reporting in future studies.

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FEVER: THE TRAVEL SOUVENIR NO ONE WANTS

Logan Spooner, Kirsten Wesolek, Gregory Altman, Rebecca Sainato

Madigan Army Medical Center, Tacoma, WA, United States

Typhoid fever should be considered in the ill returning traveler. Invasive nontyphoidal salmonellosis (NTS) is rarely associated with travel and is more often identified in vulnerable hosts with environmental exposures. Splenomegaly is a consistent feature of typhoid fever but not NTS-gastrointestinal disease. Here we present an unusual case of invasive NTS in an otherwise healthy adolescent traveler. A 16-year-old male with no past medical history presents to the emergency department with three days of fever, abdominal pain, vomiting, and non-bloody diarrhea. Nine days earlier he returned from a 4-day vacation in Punta Cana, Dominican Republic. He is ill but not toxic-appearing, and febrile (39.5ºC); labs demonstrate mild hepatitis (ALT 90U/L), elevated inflammatory markers (CRP 12.5mg/dL), and hematuria. Point-of-care ultrasound reveals splenomegaly (craniocaudal length 13.9cm). Blood and stool cultures are obtained; he receives ceftriaxone and is discharged with oral ciprofloxacin for presumed typhoid fever. Blood culture turns positive at thirteen hours; stool culture remains negative. His subsequent course is uncomplicated. Final blood culture speciation identifies *Salmonella enterica* serovar Panama. Invasive NTS is rare in high-income countries but commonly reported in sub-Saharan Africa, often in patients with malaria or HIV. In these cases, NTS disease mirrors typhoid fever, with absent diarrhea and frequent splenomegaly. *S. panama* typically causes isolated gastrointestinal disease but has a higher prevalence of invasive disease than most other NTS strains. Our case suggests splenomegaly may be a feature of invasive NTS even in healthy travelers. Ultrasound could be considered as a screening exam for this diagnosis, but not to distinguish Typhoid fever from NTS.

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DIAGNOSTIC ACCURACY OF RAPID ANTIBODY TEST COMPARED TO STOOL CULTURE FOR *SALMONELLA TYPHI* IN GHANA

Eunice Asaga[1], Denis Dekugmen Yar[1], Daniel Hayford[1], Kofi Agyapong Addo[1], Philip Adom Yeboah[2], Peter Sadari[1], Esther Ayemugah[3], Daniel Quarshie[1], Prince Kyere Dwaah[1]

[1]Akenten Appiah-Menka University of Skills Training and Entrepreneurial Development, Kumasi, Ghana, [2]Korle Bu Teaching Hospital, Accra, Ghana, [3]Greater Accra, AA, Ghana

Typhoid fever remains a major cause of febrile illness in Ghana, yet diagnosis frequently relies on rapid serological tests despite concerns about their accuracy. Evaluating the diagnostic performance of serological tests against culture-based methods is essential for improving case management and guiding public health decision-making. A cross-sectional study involving 234 patients clinically suspected to have typhoid fever was conducted at the Korle-Bu Teaching Hospital in Accra, Ghana. Each participant provided blood samples for Widal and Typhidot testing and stool samples for culture, which is the gold standard. Diagnostic performance was assessed using sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and Cohen's kappa. Logistic regression examined associations between typhoid infection and socio-demographics, symptoms, hygiene practices, and antibiotic use. Stool culture confirmed typhoid fever in 5.1% of participants. Typhidot positivity was 50.4%, whereas Widal positivity was 35.9%. Typhidot demonstrated 100% sensitivity and 52.3% specificity, whereas Widal showed 83.3% sensitivity and 67.6% specificity. Both tests exhibited low PPVs (10.2% and 12.2%, respectively) but high NPVs (>97%). Agreement between the two serological tests was moderate ($\kappa = 0.47$, $p < 0.001$). Most sociodemographic variables and clinical symptoms were not significantly associated with infection, except for a prior history of typhoid, which increased the likelihood of a positive result (AOR = 2.06, $p = 0.008$). Typhidot outperformed the Widal test in sensitivity; however, both tests demonstrated poor specificity and low predictive value compared with stool culture, limiting their reliability as standalone diagnostic tests. Strengthening laboratory capacity for culture-based methods and promoting rational antibiotic use remain critical for improving typhoid diagnosis and mitigating antimicrobial resistance in Ghana.

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CHANGING EPIDEMIOLOGY OF NON-TYPHOIDAL SALMONELLA IN NAIROBI, KENYA

Kelvin Kering, Cecilia Mbae, Samuel Kariuki

Kenya Medical Research Institute, Nairobi, Kenya

Nontyphoidal *Salmonella* (NTS) remains a prominent cause of invasive disease in sub-Saharan Africa, particularly among children under five years. Increasing resistance to critical antibiotics is likely to result in poor patient outcomes. Continued research into the transmission, evolution, and resistance patterns is critical for developing prevention and control strategies. This study investigated the circulating NTS sequence types in a disease-endemic urban informal settlement in Nairobi, Kenya. Between June 2021 and November 2024, children under five in Mukuru slums in Nairobi presenting with fever with or without diarrhoea were recruited. Blood and stool were collected and subjected to culture. NTS were subjected to antimicrobial susceptibility testing against 14 antibiotics. Index cases and healthy individuals were then followed up to check for NTS shedding. Sequencing data were used to confirm sequence type, strain relatedness. NTS prevalence was 1.5%. Among the NTS-positive cases, 93.22% (55/59) had NTS-positive stool samples, while 5.08% had NTS-positive blood samples. 50.85%, (30) of the cases were positive for *S*. Enteritidis ST11, and 21 (35.59%) *S*. Typhimurium ST19. 30.43% (14/46) of the index cases were shedding NTS post-treatment, and the isolates were closely related to isolates recovered during acute disease. Out of 74 NTS isolates from cases and healthy individuals, 47 (63.5%) were *S*. Enteritidis ST11, the rest being *S*. Typhimurium ST19. 37.8% (28/74), were resistant to at least one antimicrobial and a small proportion (8.1%) were multidrug-resistant. Resistance to azithromycin was observed in 13.5% (10/74) of the isolates. Notably, none of the isolates in this study, including those from blood, was *S*. Typhimurium ST313, an indication of the changing epidemiology of iNTS in this setting. Resistance to azithromycin is likely to impair empirical therapy in this setting. In addition, recovery of closely related isolates from cases and healthy individuals further supports the hypothesis of human-to-human transmission of NTS in sub-Saharan Africa, highlighting the possible benefit of vaccine introduction.

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DEVELOPMENT AND INTERNAL EVALUATION OF MACHINE LEARNING MODELS FOR PREDICTING MICROBIOLOGICALLY CONFIRMED SHIGELLA INFECTION IN CHILDREN UNDER FIVE USING GLOBAL ENTERIC MULTICENTRE STUDY DATA: DEVELOPMENT OF A PROTOTYPE WEB-BASED DECISION-SUPPORT TOOL

Md Fuad Al Fidah[1], Md Ridwan Islam[2], Sharika Nuzhat[2], ASG Faruque[1], Mustafa Mahfuz[1]

[1]icddr,b, Dhaka, Bangladesh, [2]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh

Shigella infection remains a major cause of diarrhoea in children under five, particularly in low- and middle-income countries (LMICs). Laboratory diagnostics are effective but often inaccessible, and dysentery-based antibiotic treatment lacks sensitivity because not all dysentery is attributable to shigellosis. Machine learning offers a potential approach to support diagnosis in resource-limited settings. This study aimed to develop and internally evaluate machine learning models to predict microbiologically confirmed Shigella infections and to demonstrate the feasibility of translating the best-performing model into a prototype web-based decision-support application. Data from 3,356 children (excluding co-infection and non-diarrhoeal children) enrolled in the Global Enteric Multicenter Study. Several machine learning algorithms (logistic regression model with all variables, penalised regression, elastic net, decision trees, random forest, gradient boosting, k-nearest neighbours, and support vector machine)

were trained and evaluated using cross-validation. Model performance was evaluated using the area under the receiver operating characteristic curve (AUC), recall, and Brier score, with recall prioritised to minimise false negatives. The most effective model was subsequently simplified using the ten most informative predictors and implemented as a prototype web-based application. All analyses were restricted to internal validation. The support vector machine (SVM) demonstrated the highest recall (0.64) while maintaining fair AUC (0.74). The parsimonious SVM model, retrained with the ten most informative predictors, achieved a recall of 0.67, AUC of 0.77, and Brier score of 0.16. A prototype web-based application was developed to demonstrate real-time predictions. This internally validated model shows potential for risk stratification using routine data; however, external validation and impact evaluation are required before clinical or operational use.

7203

ASSESSING SPATIAL RELATIONSHIPS BETWEEN LONGITUDINAL SEROCONVERSIONS AND CONFIRMED ENTERIC FEVER CASES IN URBAN AND PERI-URBAN NEPAL

Kristina W. Lai[1], Nishan Katuwal[2], Jessica Seidman[3], Shiva Ram Naga[4], Rajeev Shrestha[5], Sabin Bikram Shahi[6], Suman Shrestha[5], Krista Vaidya[7], Kate Doyle[3], Denise Garrett[3], Jason R. Andrews[8], Richelle Charles[9], Dipesh Tamrakar[10], Kristen Aiemjoy[11]

[1]University of California, Davis, Davis, CA, United States, [2]Dhulikhel Hospital Kathmandu University Hospital, Dhulikhel N.p, BA, Nepal, [3]Sabin Vaccine Institute, Washington, DC, United States, [4]Dhulikhel Hospital, Kathmandu University Hosptial, Bhaktapur, BA, Nepal, [5]Dhulikhel Hospital, Kathmandu University Hospital, Dhulikhel, Nepal, [6]Dhulikhel Hospital Kathmandu University Hospital, Dhulikhel, Nepal, [7]University of California Davis, Davis, CA, United States, [8]Stanford University, Stanford, CA, United States, [9]Massachusetts General Hospital, Boston, MA, United States, [10]Kathmandu University School of Medical Sciences, Kathmandu, Nepal, [11]University of California Davis, Fair Oaks, CA, United States

Spatial patterns of enteric fever transmission remain incompletely understood, and prior studies provide inconsistent evidence regarding the scale and context of spatial clustering across endemic settings. This analysis examines spatial clustering of seroconversions and their spatial association with blood culture confirmed enteric fever cases. Data were collected as part of a population-based longitudinal serosurvey conducted between 2019-2021 in two hospital catchment areas of Kathmandu (urban) and Kavrepalanchok (peri-urban), Nepal, and were compared to blood-culture confirmed cases from a hospital-based study in the same catchment areas from 2017-2021. Dried blood spots were collected from each individual and quantitative HlyE IgA and IgG antibody responses were measured. Geographic coordinates were collected at each individual's home during baseline study visits. Seroconversion was defined as a ≥3-fold increase above the assay detection limit and at least a 1 ELISA unit rise in IgA or IgG. Incremental Spatial Autocorrelation identified a threshold distance of significant correlation at 500m and Anselin Local Moran's I was used to assess for statistically significant clusters and spatial outliers in the population-based cohort. The spatial relationship between seroconversions and confirmed cases was assessed using both distance to the nearest confirmed cases and density of cases within 500m and modeled using log-binomial regression. A total of 749 individuals were included from the population-based cohort alongside 449 blood culture-confirmed cases. There was no significant spatial clustering of seroconversions and in log-binomial regression models, neither proximity to confirmed cases nor local case density were significant predictors of seroconversion. Overall, these findings suggest that transmission of enteric fever in urban and peri-urban Nepal likely occurs outside the immediate neighborhood area, highlighting the potential importance of shared community-level exposures such as water or food sources.

7204

WHOLE GENOME SEQUENCING UNCOVERS A CRYPTIC R6K PLASMID IN A CLINICAL *YERSINIA ENTEROCOLITICA* BIOTYPE 1A ISOLATE

Lidimarie Trujillo Rodriguez[1], Chanel Mosby-Tourtellot[1], Adrian Caroline Paskey[1], Andrea Luquette[1], Maren C. Fitzpatrick[1], Ghyssella E. Valdiviezo[1], Kyle A. Long[1], Hunter Jackson Smith[2], Mathew Esona[3], Regina Zing Cer[1], Francisco Malagon[1], Cameron Vincent Sayer[1], Francisco Mimica Porras[3], Kimberly Bishop-Lilly[1]

[1]Naval Medical Research Command, Frederick, MD, United States, [2]Global Emerging Infections Surveillance Branch, Armed Forces Health Surveillance Division, Silver Spring, MD, United States, [3]Naval Health Research Center, San Diego, CA, United States

Yersinia spp. cause yersiniosis, an acute gastroenteritis (AGE) infection and diarrheagenic disease that can progress to life-threatening sepsis. Two *Yersinia* samples from patients, including one from a military recruit, in the southwest United States presenting with AGE were collected for genetic characterization in support of Service member readiness, fitness, and capacity. The samples were streaked for isolation, and one sample had two distinct morphologies, suggesting a co-occurrence or co-infection of *Yersinia* spp. All three clinical *Yersinia* isolates were sequenced on a PacBio Vega, and the resulting assemblies were characterized for antimicrobial resistance (AMR) genes, virulence factor (VF) genes, and plasmid content. Phylogenetic analysis showed that one isolate clustered with *Y. enterocolitica* and the co-infection isolates clustered with *Y. enterocolitica* and *Y. alsatica*. The genetic characterization results for the *Y. enterocolitica* isolates suggested that these are biotype 1A. While each isolate had at least one plasmid, all AMR genes were found on the chromosomes. These AMR genes encoded multi-drug efflux pumps, beta-lactamases, and the glycopeptide resistance gene *vanG*. None of the isolates possessed the pYV virulence plasmid, while VF gene analysis found the presence of heat-stable toxin gene *ystB* in *Y. enterocolitica* isolates. Notably, the *Y. enterocolitica* from the co-infection contained an R6K-like plasmid carrying a type IV secretion system (T4SS), which we hypothesize to be used in mobilization. Further analysis of the plasmid revealed that it carried the virulence regulator genes *hha* (a hemolysin expression modulator) and *hns* (a DNA-binding protein). BLAST analysis showed the T4SS shares homology with plasmids from other *Enterobacteriaceae*, suggesting potential inter-genus plasmid transfer. In this work we characterize the AMR and VF landscape of two *Yersinia*-driven AGE cases, including a potential co-infection. Characterizing the AMR and VF landscape of current AGE-causing agents within the Military Health System supports military medical readiness against evolving threats.

7205

MULTI-STAGE MODELLING APPROACH TO ASSESS SEASONAL, CLIMATIC AND INTERVENTION EFFECTS OF DIARRHOEA AMONG UNDER-FIVE CHILDREN IN MEGHALAYA, INDIA

Riviarynthia Kharkongor

Riviarynthia Kharkongor, Shillong, India

We applied a multi-stage modelling approach to understand the effect of seasonality, environmental factors and public health interventions on monthly diarrhoeal cases (2010-2021) from Meghalaya, a northeastern state of India with a high disease burden (10.4%). First, seasonality was characterised using negative binomial regression models. Second, these models were extended to incorporate climatic variables with lag structures. Third, intervention indicators such as oral rehydration solution (ORS) distribution, zinc supplementation, and rotavirus vaccination coverage will be incorporated into the model to assess the impact on diarrhoeal disease trends. A clear seasonal pattern of diarrhoea was observed in the monthly dataset, with cases peaking around February ($p = 0.002$; peak timing 95% CI: 1.22-2.53 months). Climatic variables such as rainfall ($\beta = 0.00017$, $p = 0.59$) and temperature ($\beta = -0.030$, $p = 0.079$) in the same month were not associated with diarrhoea. However, delayed effects

such as higher rainfall one month earlier was linked to an increase in cases ($\beta = 0.00092$, $p < 0.001$), while rainfall two months earlier was not significant ($p = 0.24$). Temperature showed a mixed delayed effect: higher temperature one month earlier was linked to fewer cases ($\beta = -0.027$, $p = 0.024$), but higher temperature two months earlier was strongly linked to more cases ($\beta = 0.431$, $p < 0.001$). Additional analyses including extreme weather events and public health measures such as oral rehydration solution (ORS), zinc supplementation, and rotavirus vaccination will further help better understand these trends.This multi-stage modelling approach highlights the role of environmental effects in diarrhoeal disease dynamics. Ongoing analyses incorporating extreme weather events and public health interventions will further clarify how these factors interact. The approach provides opportunities to assess the complex interplay of multiple time-variant factors influencing diarrhoeal patterns in a comprehensive and robust manner.

7206

COMPARISON OF PRESERVATION AND ISOLATION TECHNIQUES FOR *VIBRIO CHOLERAE* WHOLE GENOME SEQUENCING

Kirabo Tess Ayazika[1], Nathaniel L. Matteson[2], Audrey Safir[1], Kelsey Murt[1], Erin Tyler Baumgartner[1], Etienne Guenou[3], Jerome Ateudjieu[3], Ketina Hirma Tchio-Nighie[3], Armand Kwetchop Dopgang[3], David Allen Sack[1], Shirlee Wohl[2], Amanda K. Debes[1]

[1]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [2]Brigham and Women's Hospital, Harvard Medical School, Boston, MA, United States, [3]Meilleur Accès aux Soins de Santé (M.A. SANTE), Yaoundé, Cameroon

Cholera caused 614,828 new cases and 7,598 deaths in 2025, disproportionately affecting areas lacking laboratory infrastructure. Whole genome sequencing (WGS) can augment outbreak surveillance by informing molecular transmission patterns and strain tracking, but current approaches rely on cultured isolates and cold chain logistics, both often infeasible in outbreak-prone settings. Culture-independent and temperature-stable preservation techniques may provide alternatives. We performed WGS on matched *Vibrio cholerae*-positive samples processed as direct stool on filter paper, rectal swabs, APW-enriched samples, and rapid diagnostic test (RDT) wicks, comparing nine alternate sample types to the gold-standard of column-purified cultured isolates. We optimized DNA extraction and library preparation for each method and assessed sensitivity (presence of *V. cholerae* reads), analytical specificity (percentage of reads mapping to the reference genome), and genome completeness (coverage, depth, and assembly metrics). A sequencing run of 96 specimens generated ~268 GB and >500 million reads. Cultured and selectively incubated samples (TCBS, APW) showed higher fraction of reads mapping to the *V. cholerae* reference genome than uncultured types, reflecting pathogen enrichment and dilution by host or microbiota DNA in uncultured specimens. Alternative sample types required 1-10 million reads for full-coverage genome assembly, roughly 2-4x the gold standard but uniquely provided metagenomic profiles of the broader microbial community, insights into within-host *V. cholerae* diversity, and greater capture of intrahost variants. Enriched sample types showed selective enrichment of specific bacterial taxa, suggesting different preservation approaches offer insights into the polymicrobial context of infection. With the exception of whole stool RDTs, most sample types supported full *V. cholerae* genome assembly, indicating broad suitability for WGS in outbreak settings.

7207

IMPACT OF KNOWN ANTIBIOTIC EXPOSURE ON CHOLERA DIAGNOSTIC TEST PERFORMANCE: A PROSPECTIVE INTERRUPTED TIME-SERIES STUDY IN BANGLADESH

Ishae Sriguha[1], Md Abu Sayeed[1], Imrul K. Nabil[2], Piyash Bhattacharjee[2], Zahid H. Khan[2], Yasmin A. Begum[2], Md. Taufiqur R. Bhuiyan[2], Mohammad A. Amin[2], Kamrul Islam[2], Firdausi Qadri[2], Ashraful I. Khan[2], **Eric J. Nelson**[1]

[1]Departments of Pediatrics and Environmental and Global Health, University of Florida, Gainesville, FL, United States, [2]Infectious Diseases Division (IDD) & Nutrition and Clinical Services Division (NCSD), International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Dhaka, Bangladesh

Diagnostic performance for *Vibrio cholerae* (*Vc*) is impacted by multiple factors, including antibiotics and virulent phage. Our objective was to quantify the effect of antibiotic exposure on diagnostic positivity and determine whether phage have an additive effect. We conducted a prospective interrupted time-series study of patients older than 2 years of age admitted with diarrheal disease at the International Centre for Diarrhoeal Disease Research, Bangladesh (Dhaka). Samples were collected only from patients with liquid stool, pre- and post-doxycycline exposure. Assays for *Vc* included culture, quantitative PCR (qPCR), and rapid diagnostic tests (RDTs); phages (ICP1,2,3) were assayed by qPCR. A total of 500 patients were enrolled. When assessed by standard of practice, 3 (1%) had no dehydration, 254 (51%) had some dehydration, and 243 (49%) had severe dehydration. When assessed with a new evidence-based scoring method, 254 (51%) had no dehydration, 69 (14%) had some dehydration, and 177 (35%) had severe dehydration. Samples positive for *Vc* by culture at 0 (pre-antibiotic), 4, and 24 hours were 226 (45%), 206 (42%), and 62 (16%), respectively. Similar values and rates were observed for *Vc* RDTs and qPCR. ICP1 was detected in 86 (17%) samples at 0 hours, ICP2 was not detected, and 8 (2%) had ICP3. Pairwise analyses found statistically significant differences in rates of culture, RDT, and qPCR *Vc* positivity pre- and post-antibiotic exposure; the effect was greatest at 24 hours. Taken together, we quantified a negative effect of known antibiotic exposure on *Vc* diagnostic positivity. The results caution against testing for cholera after antibiotic exposure to avoid false negatives. Future analyses will test whether phage predation acts as an accelerant to the effects of antibiotic exposure; the analysis will account for dehydration severity, pre-admission antibiotic exposure and time since antibiotic exposure.

7208

ANTI-*VIBRIO CHOLERAE* BACTERIOPHAGE ANTIBODY RESPONSES IN SERUM OF CHOLERA PATIENTS FROM THE DEMOCRATIC REPUBLIC OF CONGO

Tiffany N. Nguyen[1], Kilee Davis[1], Md Abu Sayeed[2], Andrew Azman[3], Eric Jorge Nelson[4], Daniel T. Leung[5]

[1]University of Utah, Salt Lake City, UT, United States, [2]MGH, Boston, MA, United States, [3]Johns Hopkins Bloomberg School of Public Health, Genève, GE, Switzerland, [4]University of Florida, Gainesville, FL, United States, [5]Univ of Utah School of Medicine, Salt Lake City, UT, United States

Current methods for cholera surveillance rely on clinical reporting with targeted microbial confirmation when indicated. This approach relies on functional health systems and bypasses non-medically attended cases. Serosurveillance overcomes these limitations and has been shown to effectively estimate cholera burden. Current antibodies used for cholera serosurveillance target known immunogenic antigens of *Vibrio cholerae*. These antibodies have limitations that include the inability to distinguish infection from vaccination and to detect infection in the first few days following exposure. Host immunity against common *V. cholerae* bacteriophages (e.g., ICP1) have not been examined for use in cholera serosurveillance. Our study aims to quantify anti-ICP1 serum antibody titers using enzyme-linked immunosorbent assay (ELISA) and assess how antibody levels change over time. Serum samples at varying timepoints (Day 0, 10, 30, and 60) following presentation to a cholera treatment center were collected from *V. cholerae* O1 rapid diagnostic test (RDT) positive patients

in Uvira, the Democratic Republic of Congo. Samples were evaluated by culture for *V. cholerae* and underwent vibriocidal antibody testing. Samples were then classified as culture-positive, negative, or indeterminate and as vibriocidal-positive or negative based on a four-fold or greater rise in vibriocidal titer within 100 days of treatment center presentation. We optimized an ELISA protocol for the major head protein of the ICP1 bacteriophage (GP122) with IgG as the secondary antibody. ELISAs were performed on patients with at least 3 of the 4 serum timepoints available. At time of abstract submission, antibody responses were obtained for 61 samples from 18 subjects. Compared to culture-negative subjects, subjects which were culture-positive and vibriocidal-positive exhibited a higher antibody response at Day 0, which waned at Day 10. Analysis of anti-GP122 responses in 244 samples from 77 subjects is ongoing. Further work is needed to evaluate the use of antibody responses to *V. cholerae* bacteriophage protein GP122 as a novel biomarker for cholera serosurveillance.

7209

TWO CASES OF OCULAR TOXOPLASMOSIS ASSOCIATED WITH INGESTION OF WHITE-TAILED DEER: EXPANDING THE SPECTRUM OF FOODBORNE ZOONOTIC TRANSMISSION IN FLORIDA, USA

Akankcha Alok, Norman L. Beatty

University of Florida, Division of Infectious Diseases and Global Medicine, Gainesville, FL, United States

White-tailed deer (*Odocoileus virginianus*) is a common animal hunted for sport and consumption in North America. The meat or 'venison' when ingested undercooked or raw can lead to acute toxoplasmosis. We highlight a unique transmission pathway by illustrating two cases of ocular toxoplasmosis in Florida associated with consumption of undercooked venison. A 61-year-old female without significant comorbidities presented with one week of new floaters with progression to near blindness in the right eye. Fundoscopic exam revealed significant subretinal white lesions consistent with retinal toxoplasmosis. *Toxoplasma gondii* serologies were elevated (IgM: 14.2 AU/mL, IgG: 374 IU/mL), indicating acute infection. The patient reported frequent consumption of undercooked venison with last use within a week. She was treated with PO trimethoprim-sulfamethoxazole (TMP-SMX) for 12-weeks, resulting in symptomatic improvement and resolution of retinal lesions on 3 and 6-months follow-ups. A 62-year-old male with hypertension and seizure disorder presented with bilateral vision changes. The patient reported frequently eating undercooked ground venison as burgers. His last ingestion was two weeks prior to the symptom onset. MRI revealed bilateral optic neuritis. Ocular toxoplasmosis was suspected and serologies returned elevated (IgM: 14.6 AU/mL, IgG: 346 IU/mL), confirming the diagnosis. He was treated with a 12-week course of PO TMP-SMX and corticosteroid taper, with eventual stabilization of vision and no new lesions. Other infectious and demyelinating etiologies were excluded in both cases. Both patients had declining anti-*T. gondii* titers after treatment. Foodborne toxoplasmosis from venison has been associated with acute toxoplasmosis but remains underrecognized in Florida. As highlighted by these cases, venison can harbor viable tissue cysts in skeletal muscles and when insufficiently cooked may lead to oral transmission. Given that white-tailed deer hunting and venison consumption are common in certain regions of Florida, heightened awareness among hunters is essential to prevent *T. gondii* infection through oral ingestion.

7210

IMPORTED SCHISTOSOMIASIS AND STRONGYLOIDIASIS AMONG MIGRANTS IN ISRAEL: PERSISTENT INFECTION AND MISSED DIAGNOSTIC OPPORTUNITIES

Talya Finn[1], Frida Babushkin[1], Keren Geller[1], Natan Shata[2], Noa Dahan[2]

[1]Laniado Hospital, Netanya, Israel, [2]NALA, Tel Aviv-Yafo, Israel

Schistosomiasis (SCH) and strongyloidiasis (SS) are neglected tropical diseases that can persist asymptomatically for decades after exposure, posing ongoing risks in non-endemic settings with migrant populations. In Israel, low clinical suspicion and nonspecific presentations may contribute to underdiagnosis. We conducted a retrospective analysis of 404 patients evaluated at Laniado Hospital who presented with eosinophilia or related symptoms and underwent serologic testing (ELISA/Western blot) for Schistosoma spp. and/or Strongyloides stercoralis. Demographic, clinical, and laboratory data were extracted, and associations between symptoms and infection status were assessed using chi-square and Fisher's exact tests. Overall, 206/404 (51%) patients tested positive for SCH and/or SS, all among immigrants from endemic regions; 55% were born in Ethiopia and approximately 10% had dual infections. Among a subset with positive schistosomiasis serology, PCR confirmed active infection in all tested cases, while stool microscopy was uniformly negative. Notably, 50% of infected individuals had resided in Israel for more than 15 years without return to endemic areas, indicating long-term persistence of infection. Gastrointestinal symptoms were significantly associated with infection, reported in approximately 40% of infected individuals compared to 25% of non-infected individuals ($p<0.05$). These findings highlight substantial underdiagnosis of chronic parasitic infections among migrants and demonstrate limitations of conventional diagnostics such as stool microscopy. Nonspecific gastrointestinal symptoms represent a missed opportunity for earlier detection. Strengthening clinician awareness, implementing targeted screening strategies, and improving surveillance systems are critical to reducing diagnostic delays and preventing long-term complications in non-endemic settings.

7211

ETHANOLIC EXTRACT OF *COLA ACUMINATA* ATTENUATES OPIOID-INDUCED RESPIRATORY DEPRESSION WITH IMPLICATIONS FOR LOW-RESOURCE CLINICAL SETTINGS

Savannah Owusu Amoako, Kwaku Appiah, Laud Arthur, Raychill Frimpomaa Acquah

University of Energy and Natural Resources, Sunyani, Ghana

Medically induced respiratory depression remains a critical challenge, particularly in low- and middle-income countries, due to opioids and sedative-hypnotics. Opioid-induced hypoventilation is life-threatening, and while naloxone can reverse respiratory depression, it may also negate analgesia and is not always accessible. This study evaluated the effects of ethanolic seed extracts of *Cola acuminata*, which contains methylxanthines (caffeine, theophylline, theobromine) known to stimulate respiration, on morphine- and pentobarbital- induced respiratory depression in rabbits (l). Seeds were air-dried, powdered, and extracted with 70% ethanol. Eight rabbits were randomly assigned to two experimental models: morphine (10 mg/kg)- and pentobarbital (25 mg/kg)-induced respiratory depression. Within each model, animals received either vehicle or C. acuminata extract at 50, 150, or 500 mg/kg. Respiratory rate was monitored every 15 minutes via abdominal movement counts. Data were analyzed using one-way ANOVA with Dunnett's post hoc test (p < 0.05). The extract significantly attenuated morphine-induced respiratory depression, with maximal effect at 500 mg/kg, but did not significantly affect pentobarbital-induced depression. These findings suggest a selective protective effect of C. acuminata against opioid-induced respiratory depression, likely mediated by its methylxanthine content. Further studies are warranted to isolate and characterize the bioactive compounds responsible for this effect.

7212

MOLECULAR CHARACTERIZATION OF WHITE GRAINS MYCETOMA CAUSATIVE AGENTS IN SENEGAL

Pape Ndiole Diagne[1], Maguette Faye[1], Pab Carole Minlekib[1], Maodo Ndiaye[2], Abdou Magip Gaye[3], Souleymane Doucoure[4], Babacar Faye[2], Cheikh Sokhna[4], Wendy WJ Van De Sande[5], Doudou Sow[1]

[1]Université Gaston Berger, SAINT-LOUIS, Senegal, [2]Université Cheikh Anta Diop, Dakar, Senegal, [3]Service d'anatomie pathologie, Hopital Fann, Dakar, Senegal, Dakar, Senegal, [4]Institut de Recherche pour le Développement (IRD), Dakar, Senegal, [5]ErasmusMC, Gilze, Netherlands

Mycetoma is a chronic inflammatory infection caused by bacteria or fungi, characterized by the discharge of black, red, white, or yellow grains. The identification of the causative agent particularly in white-grain mycetoma, remains a diagnosis challenge. This study aimed to characterize white-grain mycetoma causative agents in Senegal. We conducted a descriptive cross-sectional study from July 2022 to July 2025 in several hospitals in Dakar, Saint-Louis, Thiès, and Matam. All patients presenting with polyfistulated swelling and discharge of white grains were included. Each case underwent direct examination, culture on Sabouraud and Lowenstein media, histopathological examination, and multiplex qPCR targeting the ITS regions of fungal agents and the HSP65 gene of *Actinomadura* and *Streptomyces*. In positive cases, specific qPCR assays were performed to identify *Trematosphaeria grisea*, *Scedosporium boydii*, *Actinomadura pelletieri*, *Actinomadura madurae*, *Streptomyces somaliensis*, and *Streptomyces sudanensis*. A total of 43 patients were included, with a predominance of the inflammatory form (22.5%) and bone involvement in 65.4% of cases. Direct examination was positive in 30.23% of cases, predominantly showing thin filaments (76.92%). Culture was positive in 27.9% of cases, mainly on Lowenstein medium. Histopathology revealed an actinomycotic origin in 72% of cases, predominantly due to *Actinomadura madurae*, while 4% were of fungal origin and 24% remained undetermined. Molecular analysis identified 75% of the samples, with a predominance of the genus *Actinomadura*. White-grain mycetomas in Senegal predominantly present as inflammatory forms with frequent bone involvement. These findings highlight the predominance of actinomycotic etiology, with *Actinomadura madurae* as the main causative species.

7213

CONTRIBUTION OF ULTRASOUND-GUIDED PUNCH BIOPSY TO THE DIAGNOSIS OF MYCETOMA IN SENEGAL

Maguette Faye[1], Pape Ndiole Diagne[1], Carole Pab Minlekib[1], Babacar Thiendella Faye[2], Maodo Ndiaye[2], Abdoul Magib Gaye[2], Wendy WJ Van De Sande[3], Doudou Sow[4]

[1]Gaston Berger University, Saint Louis, Senegal, [2]Cheikh Anta Diop University, Dakar, Senegal, [3]ErasmusMC, Gilze, Netherlands, [4]Gaston Berger University, Saint-Louis, Senegal

Mycetoma is a chronic, debilitating subcutaneous infection caused by fungi or bacteria. Accurate diagnosis is essential, as treatment depends on the causative pathogen. Conventional punch biopsies (blind biopsies) have several limitations. They are invasive, sometimes imprecise due to the absence of grains in the specimen, and frequently yield negative cultures. This study evaluates the contribution of ultrasound-guided biopsy to improve sampling quality and diagnostic accuracy. A cross-sectional study was conducted between 2021 and 2025 in Senegal, in collaboration with Erasmus MC (The Netherlands). Two biopsy techniques were performed under local anesthesia (2% lidocaine) and aseptic conditions: conventional biopsy using a 5-mm punch (mean of two samples per patient) and real-time ultrasound-guided biopsy using a portable ultrasound device equipped with a high-resolution linear probe. Samples were analyzed by histopathology (10% formalin fixation), mycological examination (direct microscopy and culture), and molecular methods. A total of 161 samples were collected, including 104 ultrasound-guided and 57 conventional biopsies. Grains were detected in 76% of ultrasound-guided samples compared with 84.2% of conventional biopsies. Black grains were more frequent in ultrasound-guided samples (46.1%), while white grains predominated in conventional biopsies (35.1%). The culture positivity rate was fourfold higher with ultrasound guidance (40.4%) compared to conventional biopsy (10.5%). Histopathology was diagnostic in 44.2% and 63.2% of cases, respectively. The main pathogens identified were *Madurella mycetomatis* and *Actinomadura pelletieri*. Although both techniques allow for the detection of granules, ultrasound-guided biopsy significantly improves microbiological culture yield, thereby facilitating accurate identification of the causative agents of mycetoma.

7214

CLINICAL TRENDS OF TOXOPLASMOSIS IN PEDIATRIC PATIENTS: A 10-YEAR RETROSPECTIVE STUDY IN THE U.S. GULF COAST

Mariel Burquez, Jill Weatherhead

National School of Tropical Medicine Baylor College of Medicine, Houston, TX, United States

Toxoplasma gondii is a globally prevalent protozoan parasite capable of causing significant morbidity, particularly in congenital infections and immunocompromised hosts. In the United States, regional differences in climate, environmental exposure, and population demographics may influence transmission patterns. However, contemporary epidemiological data in pediatric populations, especially in the Gulf Coast region, remain limited. We conducted a retrospective chart review of pediatric patients diagnosed with toxoplasmosis at Texas Children's Hospital, a large tertiary care referral center serving a diverse population across the U.S. Gulf Coast, over a 10-year period. Clinical data were collected using REDCap and included demographics, clinical presentation, immune status, diagnostic modality, and classification as congenital or acquired infection. Additional variables included organ system involvement and treatment patterns. Preliminary analyses demonstrate heterogeneity in clinical presentation, ranging from asymptomatic or mild disease to severe manifestations, including central nervous system involvement. Both congenital and postnatally acquired infections were identified, with differences observed in presentation and clinical course. Ongoing analyses aim to characterize demographic patterns and potential risk factors, including geographic and socioeconomic variables, to better define regional disease trends. This study provides insight into the clinical and epidemiologic spectrum of pediatric toxoplasmosis in the Gulf Coast and may inform clinical suspicion, diagnostic strategies, and public health approaches in regions with similar environmental and demographic profiles.

7215

HEPATIC AND RENAL ALTERATIONS IN PATIENTS WITH DENGUE, MALARIA, AND LEPTOSPIROSIS IN THE COLOMBIAN CARIBBEAN

Rossana Villegas[1], Maria Fernanda Yasnot[1], Rander Antonio Ruiz[1], Onay Del Socorro Salgado[2]

[1]Universidad de Córdoba, Montería, Colombia, [2]Hospital San Jerónimo, Montería, Colombia

Acute febrile syndrome caused by dengue, malaria, and leptospirosis causes significant biochemical disorders, presenting overlapping clinical pictures that complicate differential diagnosis. This study aimed to characterize hepatic and renal alterations in patients with dengue, malaria, and leptospirosis from the Colombian Caribbean. A cross-sectional analytical study included 160 patients admitted to San Jerónimo Hospital in Montería with fever of unknown origin for less than seven days. Diagnosis required two blood samples: initially, 4 mL in an EDTA K3 tube for thick smear, peripheral blood smears, and polymerase chain reaction (PCR) for malaria, plus 6 mL without anticoagulant to obtain serum for dengue (ELISA IgM) and leptospira microagglutination. A second sample, collected 10-15 days later, was used for dengue ELISA (IgM) and leptospira microagglutination. The first serum sample was tested for transaminases (AST, ALT), alkaline phosphatase (ALP), bilirubins, albumin, total proteins, creatinine, and urea, comparing them with 30 healthy controls. Diagnoses

confirmed 51 dengue cases (ELISA-C IgM), 19 malaria cases (thick smear, nested PCR), and 11 leptospirosis cases (MAT). In dengue patients, there was a mild to moderate increase in AST (96.1%), ALT (70.5%), and ALP (57.37%), without relevant renal findings. Malaria patients presented significant bilirubin alterations (68.4%), mainly the direct fraction, along with slight transaminase increases (AST>ALT: 31.5%). Leptospirosis caused the most severe alterations, drastically affecting hepatic (transaminases 81.8%, bilirubins 81.8%) and renal (creatinine and urea, 45.4%) function of patients, compatible with classic Weil's syndrome, leading to two ICU deaths. Additionally, all three infections reduced serum albumin and total proteins, being most critical in leptospirosis (63.6% and 54.5%, respectively). In conclusion, these infections cause distinct organic alterations. Biochemical characterization is an invaluable clinical tool for predicting complications and guiding early therapeutic management.

7216

IRON OVERLOAD AND AP-1 LOSS AS TRANSCRIPTOMIC BIOMARKERS OF SICKLE CELL DISEASE SEVERITY

Amari Toussaint[1], Cecilia Botchway[1], Felix Abekah Botchway[2], William Agbozo[1], Jonathan K. Stiles[1], Adel Driss[1]

[1]Morehouse School of Medicine, Atlanta, GA, United States, [2]Univ of Ghana Medical School, Accra, Ghana

Sickle cell disease (SCD) is characterized by chronic sterile inflammation that scales with disease severity, yet the molecular determinants distinguishing mild from severe HbSS remain incompletely defined. PBMCs were obtained from a malaria-negative subset of a cohort recruited at Korle-Bu Teaching Hospital, Accra, Ghana. Ethics approval was granted by the University of Ghana and Morehouse School of Medicine IRBs; written informed consent was obtained from all participants. Seventeen participants were stratified by genotype and clinical severity: HbAA (n=8), HbSS Mild (n=3), and HbSS Severe (n=6). RNA was profiled using the NanoString nCounter Human Inflammation Panel (262 genes; NanoString Technologies, Seattle, WA). Raw counts underwent background subtraction, positive control normalization, and housekeeping gene normalization prior to log2 transformation. Spearman correlation with hemoglobin as a continuous severity measure and pairwise group comparisons were performed with Benjamini-Hochberg correction. Ninety genes correlated significantly with hemoglobin level (FDR-adjusted p value of 0.05 or less), reflecting a transcriptomic inflammatory gradient rather than a binary disease state. FTL was the strongest severity marker (rho=-0.75, FDR=0.004), with high discriminative capacity between severe HbSS and HbAA controls. JUN showed the inverse pattern (rho=+0.71, FDR=0.007), with expression preserved in milder disease and collapsed in severe patients. Supporting findings included upregulation of TLR4, HIF1A, ARG1, and NLRP3 with declining hemoglobin, and preservation of NFKB1, IFNG, and CD40LG in milder patients. These targets are consistent with upstream regulation by miR-451a and let-7i-5p previously identified in this cohort. Ferritin light chain elevation and AP-1 transcriptional loss emerge as candidate transcriptomic biomarkers of SCD severity, reflecting hemolysis-driven iron overload and adaptive stress response collapse respectively.

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NEUREGULIN-1 ADMINISTRATION ATTENUATES HEME-MEDIATED KIDNEY INJURY IN HUMANIZED SICKLE CELL MICE

William K. Agbozo, Wesley Solomon, Cecilia E. Lekpor, Solomon F. Ofori-Acquah, Jonathan K. Stiles

Morehouse School of Medicine, Atlanta, GA, United States

Acute hemolysis releases excess cell-free heme, a primary driver of multiorgan injury, particularly in the kidneys. Under heme stress, circulating levels of the cytoprotective factor Neuregulin-1 (NRG-1) decrease; notably, low plasma levels are associated with worsened kidney injury in humans. NRG-1 functions through ErbB4 receptor signaling, a key endogenous kidney-protective pathway. Given the burden of acute kidney injury (AKI) during hemolytic crises, we evaluated whether maintaining NRG-1 levels limits renal damage. Using Townes humanized sickle cell (HbSS) mice, we tested the hypothesis that NRG-1 reduces heme-induced inflammation, enhances renal cytoprotective factors, and attenuates kidney injury. Mice were treated daily with NRG-1 (5 µg/kg/day) or vehicle via intraperitoneal injection for two weeks. NRG-1 treatment significantly reduced plasma heme, lactate dehydrogenase (LDH), and pro-inflammatory cytokine levels, while increasing the proportion of circulating fetal hemoglobin-containing red blood cells (F-cells). Treatment mitigated elevations in urinary AKI biomarkers, including cystatin C and NGAL, and improved renal histopathology by reducing iron deposition, glomerular congestion, and sclerosis. Furthermore, NRG-1 enhanced renal heme oxygenase-1 (HO-1) expression and increased urinary repair biomarkers, clusterin and epidermal growth factor (EGF). Collectively, these findings provide new insight supporting the therapeutic potential of NRG-1 for mitigating acute kidney injury in sickle cell disease and other hemolytic conditions.

7218

REGIONAL SPECIES DIFFERENCES AS A SOURCE OF FALSE NEGATIVE PCR RESULTS FOR LEPTOSPIROSIS DIAGNOSIS IN THE PERUVIAN AMAZON

Sofia Zavala[1], Jack Anderson[1], Greisi Curico[2], Robert Rolfe[1], Veronica Carrion[1], Ernesto Ortiz[1], Thomas Burke[1], Tim Veldman[1], Juan Carlos Celis[2], Alberto Laguna[3], Nora Reyes[3], Christopher W. Woods[1]

[1]Duke University, Durham, NC, United States, [2]Hospital Regional de Loreto, Iquitos, Peru, [3]Instituto de Medicina Tropical Daniel Alcides Carrion, Universidad Nacional Mayor de San Marcos, Lima, Peru

Leptospirosis is a zoonotic disease of epidemic potential. Since 2023, multiple states of emergency have been declared in Peru due to the rise in leptospirosis cases. We compared the results of diagnostic assays for leptospirosis in patients with acute febrile illness. We enrolled patients with medically attendant acute febrile illness in Iquitos, Peru from 13 November 2024 through 12 May 2025. Patients with reported or documented fever without an identified focus were eligible. After informed consent was signed, acute (day 0) and convalescent (day 7-42) blood samples were obtained and shipped to Duke University for serological testing and polymerase chain reaction (PCR) using LipL32-based primers. All testing was conducted using commercially available assays (CE-marked; RUO in the United States). Concurrently, the Peruvian Instituto Nacional de Salud (INS) performed leptospirosis IgM ELISA, microscopic agglutination test (MAT), and/or 16s rRNA PCR, against local serovars on available samples as part of their routine febrile surveillance. Confirmed leptospirosis was defined as a >=4-fold rise in MAT titer from acute to convalescent samples or a single MAT titer >=1:800, or nucleic acid detection by PCR. Presumptive leptospirosis was defined as a MAT titer >=1:200 but <1:800 or a positive IgM ELISA in acute serum. All 191 participants were negative by commercial LipL32 PCR. Of the 69 concurrently tested by the Peruvian INS, 38 (55%) acute IgM ELISA were positive or indeterminate. Of these 69, only 30 had MAT performed; 18 (60%) and 8 (27%) met confirmed or presumptive leptospirosis case definition respectively. All but one were positive by *L. licerasiae* serovar Varillal. Notably, 2 were positive by 16s rRNA PCR at INS. LipL32 PCR performed poorly when compared to MAT for the diagnosis of acute leptospirosis in the Peruvian Amazon. Phylogenetic diversity of the LipL32 sequence in *L. licerasiae* serovar Varillal and primer mismatch has been described and may account for the lack of PCR detections in our acute samples. Expanded diagnostic workup of these samples is ongoing.

7219

FEBRILE ILLNESS ETIOLOGY IN RURAL MADAGASCAR: RATIONALE AND DESIGN OF A HOSPITAL-BASED STUDY

Rado J.L. Rakotonanahary[1], Haingotiana Faliharintsoa[1], Vero I. Ramanandraitsiory[1], Fiainamirindra Ralaivavikoa[1], Andritiana Tsirinomen'ny Aina[1], Jacques A. Kotchofa[1], Delphin Ratsimbazafy[2], Jocia Fenomanana[3], Andres Garchitorena[4], Matthew H. Bonds[5], Richelle C. Charles[6], Karen E. Finnegan[5], Stephen J. Popper[7]

[1]Pivot, Ranomafana Ifanadiana, Madagascar, [2]Ministry of Public Health, Ifanadiana, Madagascar, [3]Faculty of Medicine, University of Fianarantsoa, Fianarantsoa, Madagascar, [4]MIVEGEC, University of Montpellier, CNRS, IRD, Montpellier, France, [5]Department of Global Health and Social Medicine, Harvard Medical School, Boston, MA, United States, [6]Division of Infectious Diseases, Massachusetts General Hospital, Boston, MA, United States, [7]School of Public Health, University of California Berkeley, Berkeley, CA, United States

Ifanadiana is a rural and malaria-endemic district located in southeastern Madagascar. The burden of febrile illness is high; in a population of roughly 250,000, over 570,000 febrile cases were recorded by the health system between January 2002 and December 2025. Malaria rapid diagnostic tests (RDTs) are the primary diagnostic tool available throughout the health system; 546,066 malaria RDTs were recorded over the four-year period, with seasonal peaks during the rainy season January-April. The fraction of negative test results ranged from 45% at the community health level to 51% at primary care health centers and 86% at the district hospital, revealing a substantial undiagnosed burden of disease with consequences for clinical management, anti-microbial resistance, surveillance and public health planning. To begin to elucidate the etiology of non-malarial febrile illness in Ifanadiana we plan to enroll 1,200 febrile patients at the district hospital over a one-year period beginning in July 2026. We will collect serum, whole blood nucleic acid, dried blood spots, and nasal swabs from those with respiratory symptoms. Through planning sessions with local clinicians and review of recent epidemiological studies we have identified specific pathogens that will be prioritized for molecular testing at local laboratory facilities, including typhoidal Salmonella, West Nile Virus, Dengue and Chikungunya. Interim study results will be discussed by the research and clinical team on a quarterly basis, and a symposium will be held to present the project's findings. This study will provide clinicians and public health officials with information that can be used to improve clinical management and public health planning while also building an "information bank" of bio-samples and associated clinical metadata that can be drawn on for additional investigations and clinical test validation studies.

7220

ANCHORING ON POSITIVE SEROLOGIES ON A PATIENT WITH FEVER AND ANASARCA

Rayna Haque

University of North Carolina at Chapel Hill School of Medicine, Chapel Hill, NC, United States

An 83-year-old woman previously from China, living in North Carolina for 10 years, presented to the ED with several months of poor appetite, worsening fatigue, and intermittent fevers without a discernible cause. She had previously lived independently but developed profound weakness and daily subjective fevers one month prior. Initial evaluation revealed anemia, thrombocytopenia, splenomegaly, and gastric and esophageal wall thickening on imaging. Infectious disease workup revealed a positive interferon-gamma assay, positive stool antigen for Helicobacter Pylori, and a reactive Ehrlichia antibody. AFB sputum and Ehrlichia PCR were negative, and convalescent titers only showed a twofold increase. No improvement was noted after treatment with doxycycline and quadruple therapy for H. pylori. She was discharged and readmitted due to worsening weakness and new anasarca. The patient then had persistent fluctuating fevers, tachycardia, hypotension, and functional decline. Extensive infectious evaluation was unrevealing. Imaging revealed increased gastrointestinal wall thickening, pleural effusions, splenomegaly, and edema. PET-CT displayed multifocal bone marrow activity and bilateral adrenal hypermetabolism, and bone marrow biopsy revealed hypercellular marrow. No definitive evidence of infection or malignancy was seen on imaging. Her condition continued to deteriorate, eventually leading to severe lactic acidosis necessitating vasopressor support. After several conversations with the family, care was transitioned to comfort measures and the patient died while inpatient. This case highlights the challenge of interpreting positive infectious serologies in patients with constitutional symptoms. Evaluation was further complicated by language barriers, and the patient's immigrant background introduced an infectious exposure context that broadened the differential further. Findings that may indicate prior exposure can contribute to diagnostic anchoring that may have delayed appropriate imaging. Lack of response to antimicrobial therapy should prompt alternative diagnoses.

7221

FROM NIGERIA TO THE ER: SEVERE MALARIA IN A VISITING FRIENDS AND RELATIVES TRAVELER

Ericka A. Lara Ovares[1], Aneudy Núñez Peña[2]

[1]University of Minnesota, Minneapolis, MN, United States, [2]Health Partners, Saint Paul, MN, United States

Malaria remains a leading global health threat, with an estimated 249 million cases and 619,000 deaths annually, the majority due to *Plasmodium falciparum* in sub-Saharan Africa. Criteria for severe malaria includes hyperparasitemia, severe anemia, jaundice, renal failure, acidosis, coagulopathy, or neurologic impairment.A 57-year-old Nigerian female residing in the US for the past 20 years presented to clinic with 3-day history of severe headache, myalgias, arthralgias, generalized weakness, and dark urine. Upon further questioning, she reported she had returned a week ago from a month in Nigeria without pre-travel counseling. A malaria rapid screen test and blood smear were positive for *P. falciparum*, and patient was advised immediate hospital evaluation.On admission, she was febrile, tachycardic, and hypertensive. Labs showed thrombocytopenia, mild anemia, hyperbilirubinemia, and transaminitis. Initial parasitemia was 0.1% suspecting uncomplicated malaria. Atovaquone-proguanil was started as Artemisinin-based Combination Therapy (ACT) was unavailable. Within 48h, parasitemia rose to 3% with worsening cytopenias and elevated d-dimer. Due to concern for severe malaria with possible DIC, she was switched to IV artesunate with later transition to artemether-lumefantrine once parasitemia decreased to <0.1%. Patient experienced rapid improvement after this. She was discharged after four days, and follow-up labs confirmed hematologic recovery.This case highlights the diagnostic and management challenges of uncomplicated and severe malaria in non-endemic settings. US-based foreigners visiting friends and relatives (VFR) abroad are at increased risk for acquiring diseases like malaria due to close contact with local environments and lower likelihood of seeking pre-travel care. Delayed or inadequate therapy for uncomplicated malaria can allow progression to severe disease with life-threatening complications. Oral ACT is first-line treatment for uncomplicated falciparum malaria. However, ACT is expensive and has a shelf-life of 2 years making it challenging for hospitals in non-endemic areas to carry these medications.

7222

A LONGITUDINAL STUDY OF SEPSIS IN IQUITOS, PERU

Alyse Wheelock[1], M. Claudia Guezala[2], Cami Camizan Castro[2], Josh Chenoweth[3], Patrick J. Blair[3], Elizabeth Del Carmen Pezo Arellano[4], Vanessa Salazar[4], Isabel Bazan[5], Carolina L. Guevara[2]

[1]The Henry M Jackson Foundation for the Advancement of Military Medicine, Bethesda, MA, United States, [2]US Naval Medical Research Unit South, Lima, Peru, [3]The Henry M Jackson Foundation for the Advancement of Military Medicine, Bethesda, MD, United States, [4]Culmen International, LLC, Iquitos, Peru, [5]US Naval Medical Research Unit South, Lima, Peru, Iquitos, Peru

Deaths due to sepsis exceed 11 million per year globally. Although the etiologic distribution differs between high-, middle-, and limited-resource settings as well as tropical settings, sepsis literature from limited-resource settings is scarce. The Austere environments Consortium for Enhanced Sepsis Outcomes (ACESO) has established a multi-national, longitudinal

study of sepsis in austere environments with a focus on studying the host immune response to infection. In this abstract, we present preliminary descriptive data from a study site established in Iquitos, Peru. From January 2025 to June 2025, 19 participants with suspected sepsis (defined as suspected infection and meeting at least two SIRS criteria) were enrolled at two hospitals. Longitudinal data are available for 18 participants who continued participation. Participants were mostly male (16/18, 89%) and were a median age of 47 years (range 21-67). Diabetes mellitus was a common co-morbidity (7/18, 39%). Based on sequential organ failure assessment scores, sepsis-3 criteria were met at the time of enrollment for 6/18 (33%) participants. Median white blood cell count at time of enrollment was 10.3 x 10^9 (range 3.3-27.7 x 10^9) mostly with neutrophil predominance (median neutrophil percent of 70.6, range 52.1-88.9). Most common source of sepsis was skin and soft tissue infection and/or bone/joint infection (7/18, 39%). One participant died within 28 days and one participant died within three months of enrollment. Carbapenems were administered as part of antimicrobial management for 6/18 (33%) participants. Median duration of hospitalization was 9 days (range 3-31 days). From this small preliminary longitudinal cohort of sepsis in Iquitos, Peru, infectious sequelae of diabetes were prevalent and carbapenem use was common. As more research groups explore the immune response to sepsis in limited-resource settings, recognition of the evolving distribution of sepsis etiologies and local epidemiology will be critical.

7223

AN ANALYSIS OF CHEMOTHERAPEUTIC AND LOCAL TREATMENT AVENUES FOR CUTANEOUS AND MUCOCUTANEOUS LEISHMANIASIS: A SYSTEMATIC REVIEW AND META-ANALYSIS

Joy Xu[1], Sophia Beatrice Salazar[2], Aashita Doshi[3], Aaron Yim[4], Tasniah Jahan[5], Kayla Wang[6], Salah Abboushi[4], Daphne Simo[7], Clayton Rawson[8], Mrunal Munshi[9], Hassan Tariq[1], Angela Hong[4], Sanskriti Thapak[10]

[1]UCLA, Los Angeles, CA, United States, [2]Markham, ON, Canada, [3]UT Dallas, Frisco, TX, United States, [4]McMaster University, Hamilton, ON, Canada, [5]Hunter College, New York, NY, United States, [6]Stuyvesant High School, New York, NY, United States, [7]Meharry Medical College, Nashville, TN, United States, [8]Noorda College of Osteopathic Medicine, Provo, UT, United States, [9]UNC Chapel Hill, Chapel Hill, NC, United States, [10]Levine Middle College High School, Matthews, NC, United States

Cutaneous and mucocutaneous leishmaniasis are protozoan parasitic diseases that cause skin and mucosal lesions. Despite pediatric cases accounting for 50% of the global disease burden, less than 10% of leishmaniasis clinical trials focus on pediatric patients. This significant underrepresentation in treatment development underscores the need to standardize pediatric treatment protocols. Amphotericin B, while achieving a high efficacy rate of 74%, is associated with severe adverse effects, particularly organ toxicities. To counter these systemic toxicities, the WHO and the PAHO endorse localized treatments like heat therapy and cryotherapy for pediatric patients. This systematic review and meta-analysis compare the efficacy of amphotericin B with thermotherapy and cryotherapy in treating pediatric cutaneous and mucocutaneous leishmaniasis. The primary outcome assessed is the complete re-epithelialization of lesions, categorized into complete response, partial response, and failure. Secondary outcome measuring adverse effects. A comprehensive search was conducted across PubMed, Medline, Scopus, LILACS, and Cochrane Library for studies from 2010 to 2024. Screening, extraction, and quality appraisal using GRADE were performed by blinded reviewers with a third independent reconciler. Both peer-reviewed and gray literature in English were included, covering studies from the Old and New Worlds to minimize publication bias. The findings of this review indicate that thermotherapy and amphotericin achieve roughly comparable cure rates with around 80% across cohorts. Cryotherapy performed lower in mucocutaneous leishmaniasis with a cure rate of 60%, demonstrating its limitations as a monotherapy. Overall, this study aims to enhance clinical practice and guide future treatment guidelines for managing this disease in pediatric populations, especially as cases emerge in North America.

7224

IMPROVED OUTCOMES WITH SODIUM STIBOGLUCONATE PLUS PENTOXIFYLLINE IN MUCOSAL LEISHMANIASIS: A RETROSPECTIVE COHORT STUDY FROM PERU

Mercedes Sanchez-Diaz[1], Fatima Concha-Velasco[1], Andres G. Lescano[2], Braulio M. Valencia[1], Ana Ramos[1], Alejandro Llanos-Cuentas[1]

[1]Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Facultad de Salud Pública y Administración, Universidad Peruana Cayetano Heredia, Lima, Peru

Mucosal leishmaniasis (ML) is a severe form of American tegumentary leishmaniasis, characterized by chronic inflammation, progressive mucosal destruction, and significant morbidity. Pentavalent antimonials (Sb^+) remain first-line therapy; however, treatment failure occurs in up to 40% of cases. Pentoxifylline, a TNF-α inhibitor, has been proposed as an adjuvant therapy, but supporting evidence is limited. We conducted a retrospective cohort study in parasitologically confirmed adult ML patients treated between 2005 and 2013 at a national referral center in Lima, Peru. Patients received intravenous Sb^+ (20mg/kg/day IV) alone or combined with oral pentoxifylline (400mg TID) for 30 days. Clinical cure was defined as complete re-epithelialization without inflammatory signs at day 180. Disease severity was classified as mild (nasal mucosa only) or moderate (nasal plus oropharyngeal/supraglottic involvement); severe cases were excluded. Of 205 included patients, 139 received Sb^+ monotherapy and 66 combination therapy. Baseline characteristics were comparable between groups, except for sex distribution. The mean age was 41.5 ±14,8 years, 82% (168/205) were men, 81,5% (167/205) originated from jungle regions. The median disease duration was 24.6 months (IQR 11.8-71). Overall cure rates were significantly higher in the combination group compared with monotherapy (79% vs 61%; p = 0.01). After adjusting for disease severity, sex, and prior ML, combination therapy remained independently associated with a higher probability of cure (aRR 1.41, 95%CI 0.99–2.01; p<0.001). In subgroup analyses, cure rates were similar in mild cases (88.9% vs 81%, p = 0.54), but significantly higher with combination therapy in moderate disease (71.8% vs 44.7%, p = 0.01). Adverse events incidence was similar among both groups. These findings suggest that adding pentoxifylline to stibogluconate could improve outcomes in moderate ML. Larger prospective randomized trials are needed to confirm these results and to better define the role of pentoxifylline as adjuvant therapy.

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COMMUNITY ENGAGEMENT IN THE ACCEPTABILITY OF ACOZIBOROLE, A NEW SINGLE DOSE, ORAL TREATMENT FOR *GAMBIENSE* HUMAN AFRICAN TRYPANOSOMIASIS IN THE NORTHERN AND SOUTHERN UBANGI PROVINCES OF DRC

Digas Ngolo Tete[1], Florent Mbo Kuikumbi[1], Wilfried Mutombo Kalonji[1], Charles Kambo[2], Erick Mwamba Miaka[2], Chirac Bulanga[1], Sandra Rembry[3], Stephane Hugonnet[3], Junior Matangila Rika[1]

[1]Drugs for Neglected Diseases initiative, Kinshasa, Democratic Republic of the Congo, [2]National Program to combat Sleeping Sickness in the DRC, Kinshasa, Democratic Republic of the Congo, [3]Drugs for Neglected Diseases initiative, Geneva, Switzerland

The focus of the StrogHAT study (Stop Transmission of gambiense Human African Trypanosomiasis) was on the safety and epidemiological efficacy of a single dose of acoziborole administered to participants seropositive for *Trypanosoma brucei gambiense* infection, which had not been confirmed by parasitological tests, to prevent further transmission. It has been reported that the low adherence to treatment for HAT is due to lack of information, preconceived ideas, suspicion and rumors, fear of lumbar puncture, and fear of stigmatization. The aim of this work was to encourage acceptance of acoziborole treatment by conducting community activities during the implementation of the StrogHAT study. Community activities were conducted between March 2024 and December 2025 in the Northern and Southern Ubangi provinces to reassure potential StrogHAT participants

about the safety and reliability of acoziborole. Firstly, training was organized for health zone management teams, political authorities, journalists and community health workers. Posters and brochures, radio and television broadcasts, presentations in churches, and focus groups in schools were used as communication strategies in endemic villages. The overall attendance rate (total screened population/ total registered population) during screening activities increased from 78.9% (219,185/278,010) to 96.5% (329,261/341,310); 803 seropositive individuals with card agglutination and rapid diagnostic tests for HAT received treatment with acoziborole. In total, 27 churches were sensitized; 70,401 individuals were trained, of which 48% were women; and 19 schools visited, ten secondary and nine primary). No treatment refusals were recorded indicating strong community acceptance of the StrogHAT project. The awareness campaign resulted in excellent acceptance of treatment with acoziborole, as demonstrated by the significantly increased participation in screening. Communication and sensitization were decisive in building trust and ensuring participation.

7226

ACCESS TO FEXINIDAZOLE, A NEW ORAL TREATMENT AGAINST *RHODESIENSE* HUMAN AFRICAN TRYPANOSOMIASIS IN EASTERN AND SOUTHERN AFRICA

Florent Mbo[1], Olaf Valverde Mordt[2], Albert Nyembo[1], Alfred Chalira[3], Frank Shamilimo[4], Isaac Phiri[5], Eric Kitega[6], Junior Matangila Rika[1], Pere Simarro[2], Stephane Hugonnet[2]

[1]Drugs for Neglected Diseases initiative, Kinshasa, Democratic Republic of the Congo, [2]Drugs for Neglected Diseases initiative, Geneva, Switzerland, [3]Community Health Sciences Unit, Ministry of Health, Lilongwe, Malawi, [4]Public Health Department - NTDs Unit, Ministry of Health, Lusaka, Zambia, [5]Ministry of Health and Child Care, Harare, Zimbabwe, [6]Ministry of Health, Kampala, Uganda

The World Health Organization has set a goal to eliminate *T.b rhodesiense* human African trypanosomiasis (r-HAT) as a public health problem by 2030. Historically, treatment options for r-HAT were complex, using melarsoprol for the neuro-encephalitic stage of the disease and requiring hospitalization and specialized nursing care. The Drugs for Neglected Diseases initiative developed fexinidazole, a new oral treatment available to treat all stages of the disease, which received a positive scientific opinion from the European Medicines Agency in 2023. Over the last two years, we have been implementing the updated WHO HAT treatment guidelines in endemic countries in Eastern and Southern Africa. In this presentation, we will describe ongoing activities to promote access to treatment with fexinidazole. These have been made possible because of support given to countries through training of trainers, advocacy to ensure that authorization of use and drug request letters were sent to the WHO, and training of health care providers. In addition, training on r-HAT diagnosis has been organized in these countries to boost detection rates. The National Pharmacovigilance Systems of endemic countries have been reinforced thanks to online training provided by the Rabat Collaborating Center for Pharmacovigilance Practices (Morocco). From 2024, when fexinidazole started to be supplied in each endemic country, until the end of January 2026, 62% (28/45) of all r-HAT patients diagnosed and treated during this period were treated with fexinidazole. 5/6 countries have been using the r-HAT WHO guidelines, and 140 health care providers have been trained on r-HAT diagnosis, drug administration, and pharmacovigilance. The introduction of fexinidazole for the treatment of both stages of r-HAT is an important milestone on the road to r-HAT elimination. In addition, since staging of the disease is not required, the use of lumbar puncture has been reduced making diagnosis simpler and less of a barrier for patients.

7227

EFFICACY AND SAFETY OF LXE408, A NOVEL ORAL TREATMENT CANDIDATE FOR VISCERAL LEISHMANIASIS: RESULTS FROM A PHASE II STUDY IN ETHIOPIA (NCT05957978)

Mezgebu Silamsaw[1], **Eleni Ayele**[1], Yonael Mulat Eneyew[1], Tigist Mekonnen[1], Alexandra Solomos[2], Kirsten Carter[3], Rashidkhan Pathan[4], Gerhild Angyalosi[3], Fabiana Piovesan Alves[2], Gláucia Cota[5]

[1]Leishmaniasis Research and Treatment Center, University of Gondar, Gondor, Ethiopia, [2]Drugs for Neglected Diseases initiative, Geneva, Switzerland, [3]Novartis Pharma AG, Basel, Switzerland, [4]Novartis Healthcare Private Limited, Hyderabad, India, [5]Drugs for Neglected Diseases initiative, Rio de Janeiro, Brazil

Visceral leishmaniasis (VL) remains a major public health challenge in East Africa, where treatment options are limited and require prolonged parenteral regimens with variable efficacy and tolerability. There is a critical need for safe, effective, and short-course oral therapies to support VL elimination in the region. LXE408 is a novel oral drug candidate, the first oral selective inhibitor of kinetoplastid proteasome, active on all tested *Leishmania* species, with the potential to be effective, safer, and more convenient than existing therapies across endemic areas. A randomized, Phase II trial with an additional calibrator arm for contextual interpretation was conducted in Ethiopia to evaluate the efficacy, safety, and pharmacokinetics of LXE408 in patients with VL. In total, 52 participants were enrolled and randomized (3:1) to receive LXE408 300 mg orally once daily for 14 days (n= 39) or the standard of care regimen injectable sodium stibogluconate plus paromomycin 17 days (SSG/PM) (n=13). The primary endpoint was initial cure at Day 28, defined as clinical improvement, parasitological clearance, and no rescue treatment. Secondary endpoints included frequency and severity of adverse events, definitive cure at Day 180 and pharmacokinetics parameters measuring exposure to the drug. Recruitment was completed in May 2025, and the last patient last visit was conducted in November 2025. All participants had primary, non-severe VL confirmed parasitologically; all were male, aged 19-31 years, and were reflective of the typical VL population in the Gondar region. Clinical database lock was achieved in March 2026 and final results will be presented at the congress. This study will provide important data on the efficacy, safety and pharmacokinetics of LXE408 in the Eastern African context. Data generated in this study will further inform the design of subsequent trials, supporting the further development of LXE408, as a promising treatment candidate for patients with primary VL, a deadly disease with high unmet medical need.

7228

TRANSFORMING TREATMENT OF VISCERAL LEISHMANIASIS: A PROOF-OF-CONCEPT STUDY OF LXE408, A NOVEL ORAL TREATMENT CANDIDATE FOR VISCERAL LEISHMANIASIS, IN INDIA

Shyam Sundar[1], Krishna Pandey[2], Gláucia Cota[3], Gwenaelle Carn[4], Sheeraz Raja[5], Jaya Chakravarty[6], Neha Agrawal[7], Roshan K. Topno[2], Major K. Madhukar[2], Anurag Pappu[2], Kirsten Carter[8], Rashidkhan Pathan[9], Gerhild Angyalosi[8], Fabiana Piovesan Alves[4]

[1]Institute of Medical Sciences, Banaras Hindu University, Varanasi, India, [2]Rajendra Memorial Research Institute of Medical Sciences, Patna, India, [3]Drugs for Neglected Diseases initiative, Rio de Janeiro, Brazil, [4]Drugs for Neglected Diseases initiative, Geneva, Switzerland, [5]Drugs for Neglected Diseases initiative, New Delhi, India, [6]Patna Medical College and Hospital, Patna, India, [7]University of Florida, Jacksonville, FL, United States, [8]Novartis Pharma AG, Basel, Switzerland, [9]Novartis Healthcare Private Limited, Hyderabad, India

The kala-azar (visceral leishmaniasis, VL) elimination program (KAEP) in South Asia, including India, Nepal and Bangladesh, has achieved its goal of reducing cases to <1/10000 population, relying mainly on single-dose intravenous liposomal amphotericin B (LAmB). Short-course oral therapies adapted to integrated models are now needed to sustain these gains. This is the first study testing efficacy and safety of LXE408, a first in class oral

kinetoplastid-selective proteasome inhibitor, in VL patients. This Phase II trial was conducted in Bihar, India. 95 adult patients with VL were randomized (2:2:1) to blinded investigational study arms of LXE408 300 mg once daily for 7 (n=38) or 14 (n=38) days, or to an open label calibrator arm with single-dose LAmB 10 mg/kg (n=19). The primary endpoint was initial cure at Day 28, defined as clinical improvement, parasitological clearance, and no rescue treatment. Secondary endpoints included frequency and severity of adverse events (AEs), PK, and efficacy measured as definitive cure at Day 180. Initial cure was 100% in both LXE408 arms. Definitive cure was 94.4% (34/36; 90% CI: 88.2-100) in the 7-day and 100% (38/38) in the 14-day arms. In the LAmB arm, initial and definitive cure rates were 94.1% (16/17 90% CI: 84.7-100) and 93.3% (14/15 90% CI: 82.7-100), respectively. Treatment-emergent AEs occurred in 42.1% and 39.5% of patients in the LXE408 7- and 14-day arms, respectively and in 68.4% in the LAmB arm; with ≥grade 3 AEs reported in 10.5% (4/38) and in 7.9% (3/38) of patients in the LXE408 7- and 14-day arms, respectively and in 10.5% (2/19) in the LAmB Arm. Drug-related AEs occurred in 10-13% of participants receiving LXE408 for 14 and 7 days respectively and in 47% receiving LAmB. No reported AEs led to treatment discontinuation. Three serious AEs (including two deaths) were reported in LXE408 arms, none considered treatment-related by the Investigator. Both LXE408 regimens demonstrated high efficacy and were well tolerated, with no new safety signals identified. These findings support further development of LXE408 as a promising oral treatment for VL, particularly for sustaining the achievements of KAEP.

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SAFETY OF ACOZIBOROLE IN SEROPOSITIVE BUT PARASITOLOGICALLY UNCONFIRMED *GAMBIENSE* HUMAN AFRICAN TRYPANOSOMIASIS: RESULTS FROM A MULTICENTRE RANDOMIZED DOUBLE-BLIND PLACEBO-CONTROLLED TRIAL

Junior Matangila[1], Adeline Prêtre[2], Digas Ngolo Tete[1], Guylain Mandula[1], Florent Mbo[1], Wilfried Mutombo Kalonji[1], Mwamba Eric[3], Chirac Bulanga[1], Sandra Rembry[2], Anne Reymondier[2], Beate Aurich[2], Jean-Yves Gillon[2], Stephanie Braillard[2], Seveine Blesson[2], Antoine Joseph Tarral[2], Olaf Valverde Mordt[2], Stephane Hugonnet[2]

[1]Drugs for Neglected Diseases initiative, Kinshasa, Democratic Republic of the Congo, [2]Drugs for Neglected Diseases initiative, Geneva, Switzerland, [3]National Program to combat Sleeping Sickness in the DRC, Kinshasa, Democratic Republic of the Congo

Current treatment for *gambiense* human African trypanosomiasis (*g*-HAT) requires parasitological confirmation, leaving many seropositive individuals untreated and potentially sustaining transmission. Acoziborole, a single-dose oral benzoxaborole, could enable simplified treatment if proven safe for individuals seropositive for *g*-HAT but parasitologically negative. A multicentre, randomized, double-blind, placebo-controlled Phase II/III trial was conducted in the Democratic Republic of the Congo and Guinea. Participants aged ≥15 years who were seropositive for *g*-HAT but parasitologically negative were randomized 3:1 to receive acoziborole (960 mg) or placebo and followed for four months. The primary endpoint was the occurrence of uncommon treatment-emergent adverse events (TEAEs). Between December 2021 and August 2023, 1,208 participants were enrolled (907 received acoziborole, 301 placebo), with 99% completing follow-up. Mean age was 37 years; 53% were female. TEAEs occurred in 21.5% of acoziborole recipients and 23% placebo recipients. Most events were mild or moderate. Investigators considered 6.5% of participants in the acoziborole arm and 5.7% in the placebo arm to have TEAEs related to study drug. Serious adverse events were infrequent (0.6%) and none were reported as treatment-related. Headache was the only TEAE reported more frequently with acoziborole (3.1 vs 1.7%). A mean ΔΔQTcF shortening of -11.5 ms was observed in acoziborole group when compared with baseline and placebo. No cardiac arrhythmia was reported in association with QTcF shortening. No participants became parasitologically positive during the four-month follow-up. Acoziborole demonstrated a favorable safety profile in individuals seropositive for g-HAT but parasitologically negative. Headaches were identified as a non-serious adverse drug reaction. Arrhythmia due to QT shortening was a potential important risk, the clinical significance of which is currently unclear. These findings support the potential expansion of treatment this population, which could simplify diagnostic requirements and accelerate elimination strategies.

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NOVEL THERAPEUTIC DISCOVERY FOR CHAGAS DISEASE

Ana C. Mengarda[1], Maxim Totrov[2], Paul R. Carlier[3], R. Drew Etheridge[4], Ariel M. Silber[1], M. Belen Cassera[5]

[1]University of São Paulo, São Paulo, Brazil, [2]Molsoft LLC, San Diego, CA, United States, [3]University of Illinois Chicago, Chicago, IL, United States, [4]Univ of Georgia, Athens, GA, United States, [5]University of Georgia, Athens, GA, United States

Chagas disease affects 6-7 million people and only two drugs are available for the treatment of the disease, nifurtimox and benznidazole. Both drugs present limitations regarding efficacy and tolerance. In this context, there is an urgency to find new molecules with anti-*Trypanosoma cruzi* potential. As a starting point for the development of novel therapies with trypanocidal activity, we screened a proprietary compound library composed of chemical scaffolds that are both orally bioavailable and exhibit low toxicity. To assess the effect of compounds on *T. cruzi*, the proliferation of epimastigotes was first measured at 1 µM followed by dose-dependent assessment of active compounds for EC_{50} determination. During an initial screening with a select group of β-carbolines, PRC1901 demonstrated inhibition of epimastigote proliferation, achieving an EC_{50} value of 0.12 µM and exhibiting approximately 99.9% inhibition at a concentration of 1 µM. PRC1901 also demonstrated moderate toxicity in CHO-K_1 cells with a CC_{50} value of 3.5 µM using the MTT assay. To assess the effect on cell-released trypomastigote forms, PRC1901 was evaluated in a dose-dependent manner during the infection process and against CHO-K_1 infected cells. Trypomastigote invasion was inhibited with an EC_{50} of 0.08 µM, while the release of trypomastigotes was inhibited at an EC_{50} of 0.13 µM. This resulted in selectivity indices greater than 40 and greater than 20, respectively. The effect of PRC1901 on host cell infection and amastigote proliferation was also evaluated. CHO-K_1 host cells treated with 0.13 µM of PRC1901 were infected by trypomastigotes and amastigote proliferation was assessed. PRC1901 inhibited the replication of intracellular amastigotes as well reduced the total number of infected cells by 96.8 and 88.3% respectively. Collectively, our findings indicate that PRC1901 demonstrates potential anti-*T. cruzi* activity, and subsequent investigations will be conducted to elucidate its mechanism of action. At present, we are broadening the screening process to encompass additional scaffolds in order to identify further candidates suitable for optimization.

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AI-DRIVEN DRUG REPURPOSING IDENTIFIES A NOVEL CLASS OF HIGHLY POTENT ANTI-LEISHMANIAL COMPOUNDS WITH MILTEFOSINE-LIKE POTENCY

Rafeh Oualha, Yosser Zina Abdelkrim, Khadija Essafi-Benkhadir, Ikram Guizani, Emna Harigua-Souiai

Institut Pasteur de Tunis, Tunis, Tunisia

Leishmaniases remain a major neglected tropical disease with limited therapeutic options. Challenges including high toxicity, costs, and emerging resistance underscore the urgent need for new treatments. Drug repurposing offers a time- and cost-effective strategy to accelerate the identification of new anti-leishmanial agents, as exemplified by miltefosine, the first oral drug approved for this disease. In this study, we applied a deep learning-based drug repurposing pipeline to identify FDA-approved molecules with potential anti-leishmanial activity. Selected candidates were evaluated *in vitro* against *Leishmania infantum* and *Leishmania major* promastigotes using an MTT assay. Hits were subsequently validated against intracellular amastigotes. To assess the selectivity index, cytotoxicity was also evaluated on THP-1-derived macrophages. Interestingly, the AI pipeline identified a previously unreported chemical class of compounds, comprising three structurally related molecules. Notably, two compounds demonstrated high potency, with IC_{50} values below 4 µM against both

promastigote and intracellular amastigote stages. These values are comparable to those of the reference drug miltefosine in our experimental models. Cytotoxicity assays confirmed a favorable safety profile, with high selectivity for the Leishmania parasites relative to host macrophages. Overall, our findings highlighted the power of AI-driven methods, combined with experimental validation, in identifying three highly potent and promising a new class of anti-leishmanial candidates. Ongoing studies are focused on further characterizing the mechanism of action and evaluating the therapeutic efficacy of this chemical class of compounds.

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DESIGN OF EXPERIMENTS-GUIDED OPTIMIZATION OF A MINIATURIZED ASSAY FOR STUDYING MTAP DIFFERENTIAL EXPRESSION ACROSS PARASITE GROWTH PHASES IN *LEISHMANIA MAJOR*

Yosser Zina Abdelkrim, Sonia Abbess, Imen Gharbi, Isleme Khalfaoui, Imen Bassoumi-Jamoussi, Ikram Guizani
Institut Pasteur De Tunis, Tunis, Tunisia

5′-Methylthioadenosine phosphorylase (MTAP) is a key enzyme in the purine salvage pathway of *Leishmania major* and a potential target for drug discovery. This study aims to establish robust assay conditions transferable from recombinant protein systems to parasite extracts, for the reliable quantification of MTAP activity in parasite extracts to investigate expression profiles of this enzyme across parasite growth phases and stages. We report the development and optimization of a miniaturized 96-well enzymatic assay to measure MTAP activity in promastigotes culture extracts. The assay was adapted from previously established conditions for recombinant MTAP from *Leishmania infantum* and further optimized for *L. major* using a factorial design approach. Key parameters, including buffer composition, pH, substrate (MTA) concentration, incubation time, and enzyme source (recombinant vs. soluble parasite extract), were systematically varied. A reduced factorial design (2^{8-4}) generated a set of experimental combinations to identify conditions maximizing signal, linearity, and reproducibility. MTAP activity was monitored spectrophotometrically at 305 nm through coupling of adenine production to its equimolar conversion into 2,8-dihydroxyadenine by xanthine oxidase. The miniaturized format enables efficient testing of multiple conditions using limited biological material. Preliminary results indicate that the DoE-guided assay provides a scalable platform for MTAP activity measurement in *Leishmania major*, with potential applications in functional studies and early-stage drug discovery. Experimental validation and statistical analysis are currently ongoing.

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CHARACTERIZING PHARMACODYNAMICS OF TAFENOQUINE IN HIGH PARASITEMIA *BABESIA MICROTI* IMMUNOCOMPROMISED MOUSE MODEL

Sandra Massih[1], Isaiah Hoffland[1], Rubayet Elahi[1], Matthew Michael Ippolito[2], David J. Sullivan[3]
[1]Johns Hopkins University, Baltimore, MD, United States, [2]Johns Hopkins University School of Medicine, Baltimore, MD, United States, [3]John Hopkins Univ, Baltimore, MD, United States

There are more than 2000 annual cases of babesiosis in the United States. The first line treatment for *Babesia microti* is atovaquone with azithromycin, although atovaquone and macrolide resistance has been frequently reported in immunocompromised patients. Tafenoquine at malaria prophylaxis doses has been used in several cases of recurring *Babesia microti* with positive outcomes. In this mouse study, we compared dose-dependent or time above inhibition concentration clearance and cure for tafenoquine in atovaquone-sensitive and sequencing-confirmed atovaquone-resistant *Babesia microti* in immunocompromised mouse model. Single dose 30 mg/kg tafenoquine cures atovaquone-sensitive *Babesia*. Here we compared a single-dose 30mg/kg, 10 mg/kg q48h for three doses, and 5 mg/kg q48h for six doses. In all treatment groups, we noted a parasite vacuolation stress response occurring before parasitemia decrease. In both atovaquone-sensitive and resistant *Babesia*, the higher dose coincided with more rapid clearance. The atovaquone-sensitive mice cleared the parasites in 48-, 72-, and 155-hours post dose for the 30 mg/kg, 10 mg/kg, and 5mg/kg doses, respectively, while atovaquone-resistant mice cleared all by 96-hours post dose. Interestingly, tafenoquine was curative at all doses tested except 10 mg/kg in atovaquone-sensitive parasites, but only curative in 5mg/kg for atovaquone-resistant parasites. The translational implications of these findings are that malaria prophylaxis low doses for a longer duration schedule may be adequate for treating both atovaquone-sensitive and resistant parasites.

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PILOT PHASE 2 TRIAL TO CHARACTERIZE DOSE-RESPONSE RELATIONSHIPS FOR DRUGS IN ADULT PATIENTS WITH INDETERMINATE CHAGAS DISEASE: INTERIM ANALYSIS OF THE CHARM TRIAL

Cintia Valeria Cruz[1], James Watson[2], Israel Molina Romero[3]
[1]Mahidol Oxford Tropical Research Unit, Bangkok, ON, Canada, [2]University of Oxford, Oxford, United Kingdom, [3]Hospital Universitari Vall d'Hebron, Barcelona, Spain

Early pharmacodynamic evaluation of Chagas disease therapies is limited by the absence of validated biomarkers and by the complex within-host dynamics of *Trypanosoma cruzi*. The CHARM study is a phase 2 adaptive pharmacometric trial in Brazil designed to characterize parasite dynamics and inform dose optimization of much-needed new treatments. Adults with chronic *T. cruzi* infection and baseline parasitaemia ≥2 parasite equivalents/mL undergo one month of twice-weekly baseline qPCR sampling, followed by adaptive open-label subcurative treatment with benznidazole (100-200 mg), nifurtimox (120-600 mg), or posaconazole (600 mg) and intensive post-dose qPCR sampling; the stage 2 primary endpoint is parasite clearance half-life. We analyzed interim qPCR data from the first 12 participants.Twelve (12) adults were included, 6/12 (50%) female, with a median age of 66 years (IQR 65.5-70.3; range 47-79). Baseline parasitaemia demonstrated substantial inter-individual variability, with a median Ct of 35.7 (IQR 34.1-36.1; range 32.0-37.2), while remaining relatively stable within individuals (median within-patient coefficient of variation 5.2%, IQR 3.8%-5.7%). These findings are consistent with a near steady-state dynamic previously described and support the view that single time-point measurements are insufficient to characterize baseline parasite burden. This provides essential information for individualized pharmacokinetic-pharmacodynamic model fitting, allowing natural variability to be distinguished from drug-induced effects. As expected in this exploratory study, no consistent pharmacodynamic signal on parasite clearance has yet been detected. However, these early data establish the feasibility and scientific value of high-frequency qPCR sampling and provide a robust framework for subsequent model-based analyses of treatment response. This approach has the potential to improve the design and interpretation of phase 2 trials in Chagas disease.

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EVALUATION OF MONTELUKAST EFFECTS ON CYSTEINYL LEUKOTRIENE TYPE 1 RECEPTOR AND CARDIAC FIBROSIS REDUCTION IN CHAGAS DISEASE: A MURINE MODEL STUDY

Josselyn Karina Vaca[1], Manuela Renee Verastegui[2]
[1]Universidad Peruana Cayetano Heredia, Peru, Peru, [2]Universidad Peruana Cayetano Heredia, Lima, Peru

Chagas disease, caused by *Trypanosoma cruzi*, is a major parasitic infection with severe cardiac complications. BALBc mice are infected with *T. cruzi* and parasitemia is monitored until decline in the indeterminate phase, when animals receive montelukast treatment for one month. At eight months post infection, mice are sacrificed and cardiac tissue collected. Histological evaluation includes hematoxylin and eosin staining for tissue integrity and Masson's trichrome for fibrosis. Immunohistochemistry for CD68 is performed to assess macrophage infiltration, and cysteinyl leukotriene type 1 receptor expression is analyzed to explore drug

mechanisms. The hypothesis is that montelukast reduces cardiac fibrosis by 20%, potentially via modulation of leukotriene mediated inflammatory pathways. Ongoing analyses are expected to confirm this and provide insight into the role of leukotriene receptor antagonists in mitigating chronic cardiac pathology in Chagas disease.

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IMPROVING PENTAMIDINE THERAPY FOR CUTANEOUS LEISHMANIASIS THROUGH POLYMERIC MICELLES AND NANOFIBER-BASED DRUG DELIVERY SYSTEMS

Paul Nguewa[1], Nerea Guembe-Michel[2], Kristell Panta Quezada[1], Javier Carriles[2], Paolo Edoardo Ginatta[1], Gustavo González-Gaitano[2]

[1]University of Navarra. Department of Microbiology and Parasitology, Pamplona. Navarra, Spain, [2]University of Navarra. Department of Chemistry, School of Science, Pamplona. Navarra, Spain

Cutaneous leishmaniasis (CL) remains a neglected tropical disease with limited therapeutic options, often constrained by systemic toxicity and suboptimal drug delivery. Pentamidine (PTM), although effective, is associated with severe adverse effects that restrict its clinical use. In this context, two nanotechnological approaches have been explored to improve PTM delivery and therapeutic performance. On one hand, polymeric micelles based on Pluronic® F127 were developed as nanoscale carriers capable of enhancing PTM solubility, stability, and bioavailability. Physicochemical characterization confirmed the formation of stable micellar systems, while biological assays demonstrated a significant reduction in cytotoxicity in macrophages without compromising antileishmanial activity. Gene expression analyses further revealed modulation of parasite pathways associated with oxidative stress, calcium homeostasis, apoptosis-like responses, and drug resistance. On the other hand, pentamidine-loaded polycaprolactone (PCL) nanofibers produced by solution blow spinning (SBS) were designed as a topical delivery system. These nanofibers enabled sustained drug release and maintained potent leishmanicidal activity against *Leishmania major*. Structural analyses indicated drug-polymer interactions and controlled dispersion within the polymer matrix. At the molecular level, treatment with PTM-loaded nanofibers was associated with downregulation of genes involved in cell cycle progression and DNA replication, alongside upregulation of drug response mechanisms. Together, these studies highlight the potential of advanced nanocarrier systems, including polymeric micelles and nanofibrous matrices, to improve the therapeutic index of PTM. By reducing toxicity, enabling controlled release, and modulating parasite responses, these platforms represent promising strategies for safer and more effective topical treatment of CL.

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ADVANCED NANOCARRIERS FOR PENTAMIDINE: ENHANCING SAFETY AND ANTILEISHMANIAL EFFICACY IN CUTANEOUS LEISHMANIASIS

Paul Nguewa[1], Kristell Panta Quezada[1], Nerea Guembe-Michel[2], Javier Carriles[2], Paolo Edoardo Ginatta[1], Gustavo González-Gaitano[2]

[1]University of Navarra. Department of Microbiology and Parasitology, Pamplona. Navarra, Spain, [2]University of Navarra. Department of Chemistry, School of Science, Pamplona. Navarra, Spain

Cutaneous leishmaniasis (CL) is a global infectious disease for which current treatments remain limited by systemic toxicity and complex administration regimens. Pentamidine (PTM), although clinically effective, is associated with severe adverse effects that restrict its therapeutic use. In this study, polymeric micelles based on Pluronic F127 (F127) were developed as nanocarriers to improve PTM delivery, enhance its safety profile, and preserve antileishmanial efficacy. A comprehensive characterization combining dynamic light scattering, UV-visible spectroscopy, fluorescence, and nuclear magnetic resonance confirmed the formation of stable micellar systems, PTM-polymer interactions, and temperature-dependent self-assembly. The resulting PTM-loaded micelles exhibited nanoscale dimensions and remained stable under physiologically relevant conditions. Biological evaluation demonstrated that micellar encapsulation significantly reduced PTM cytotoxicity in RAW264.7 macrophages while maintaining potent activity against *Leishmania major* promastigotes. Furthermore, RT-qPCR analysis revealed that treatment with PTM-loaded micelles modulated parasite pathways associated with redox balance, oxidative stress, calcium homeostasis, apoptosis-like processes, and drug resistance. These findings suggest that micellar delivery not only improves PTM bioavailability but also influences parasite stress responses at the molecular level. Overall, the incorporation of PTM into F127 polymeric micelles led to a marked improvement in its therapeutic index in vitro. This nanotechnological approach represents a promising strategy to enhance the safety and efficacy of PTM, supporting its potential application in the treatment of cutaneous leishmaniasis.

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PROGRESS TOWARDS THE CREATION OF PROTAC TARGETED PROTEIN DEGRADERS FOR TRYPANOSOMAL INFECTION

Lucía Alejandra Luna Wong, Zhongsheng Zhang, John R. Gillespie, Aisha Mushtaq, Sayaka Shibata, Domnita V. Rusnac, Ning Zheng, Erkang Fan, Frederick Buckner

University of Washington, Seattle, WA, United States

Targeted protein degradation (TPD) is a growing field for cancer therapeutics that has not yet been exploited in antiparasitic drug discovery. TPD can be mediated by proteolysis-targeting chimeras (PROTACs), which are heterobifunctional small molecules formed by linking 2 ligands via a linker. One ligand interacts with a protein of interest (POI) while the other engages an E3 ubiquitin ligase. When a PROTAC simultaneously binds the POI and the E3 ligase, the complex induces POI ubiquitylation, leading to its degradation by the ubiquitin-proteasome system. PROTACs were synthesized to target 2 distinct POIs in *Trypanosoma cruzi* and *T. brucei* methionyl-tRNA synthetase (MetRS) and methionine aminopeptidase 2 (MAP2). Ligands to these proteins were conjugated by a linker to pomalidomide, used for binding to the E3-ligase cereblon. While trypanosomes lack a full-length cereblon, they encode a CULT domain protein of unknown function that may interact with pomalidomide. When tested on *T. cruzi* amastigotes, PROTACs were more potent than the unlinked target binders or pomalidomide alone, suggesting antiparasitic activity through a distinct mechanism. To further measure MAP2 degradation, a fluorophore was fused to the MAP2 gene using CRISPR technology. Trypomastigotes were then exposed to MAP2-targeting PROTACs and to the unlinked target binders. Increased fluorescence – signaling excess MAP2 protein – was observed in parasites exposed to the PROTACs, rather than the decreased fluorescence expected with MAP2 degradation. The fluorescence signal increased in a dose-response correlation to the PROTAC concentration. Next steps: 1) expression of *T. brucei* DCAF1 – present in trypanosomes – as an alternative candidate E3 ligase for PROTAC targeting. 2) Tagging of the DCAF1 gene with a luciferase tagging system for thermal shift assays to measure protein stability and infer binding of the PROTAC to the DCAF1. 3) Determine the causes of the increased mNeonGreen-MAP2 protein levels by our pomalidomide-based PROTACs. The results of these investigations will shape the next phase of this research on novel therapeutics for neglected tropical diseases.

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THE LEISHMANIASES IN ETHIOPIA: A SCOPING REVIEW SUB-ANALYSIS OF TREATMENT AND DIAGNOSTICS

Riley Ann Fanning-Hughes[1], Catherine Wenger[2], Philip Espinola Coombs[3], Katherine O'Brien[4], Adriana Totten[5], Sofia Wojtaszek[1], Ermias Diro Ejara[6], Richard G. Wamai[7]

[1]Department of Biology, Department of Psychology, College of Science, Northeastern University, Boston, MA, United States, [2]Department of Public Health, Yale University, New Haven, CT, United States, [3]Northeastern University Library, Northeastern University, Boston, MA, United States, [4]Department of Environmental and Occupational Health, Milken Institute

School of Public Health, George Washington University, Washington, DC, United States, [5]Department of Public Health and Health Sciences, Bouvé College of Health Sciences, Northeastern University, Boston, MA, United States, [6]University of Washington, Seattle, WA, United States, [7]Department of Cultures, Societies, and Global Studies, College of Social Sciences and Humanities, Northeastern University, Boston, MA, United States

The leishmaniasis are a group of neglected tropical diseases that disproportionately affect individuals living in poverty and can be fatal if left untreated. Ethiopia is an endemic region for both cutaneous and visceral leishmaniasis, but significant research gaps remain, particularly in treatment and diagnostics. In an effort to examine the current research landscape of leishmaniasis in Ethiopia, a 2025 scoping review conducted by this team identified trends in study themes, study types, and designs across 639 included articles and identified research gaps to be prioritized in future research. This study aims to further analyze the fields of diagnostics and treatment for leishmaniasis in Ethiopia, building on this prior work. This review follows JBI scoping review methodology and PRISMA-ScR reporting guidelines. The database search was re-run in February 2026 to capture new articles published in the time since the original database search (early 2024). A total of 1,719 articles were identified. A dual-blind screening process with conflict resolution was used for the abstract screen and will be used for the full-text and data extraction phases. Currently, 202 studies are included in the full-text screen, which is ongoing. The project is expected to be completed by summer 2026. Two separate analyses will be conducted for diagnostics and treatment using topic-specific variables. Identifying diagnostic and treatment gaps will guide future research and surveillance, as current tools remain limited, expensive, and often inaccessible. These findings provide an evidence base for key stakeholders, including the Ethiopian Ministry of Health, to prioritize interventions and attract targeted funding toward the populations of greatest need. Ultimately, this review supports the broader NTD community in working toward the 2030 WHO roadmap goals.

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THE MICROBIOME OF BURULI ULCER AND ITS IMPACT ON INTERVENTION OUTCOMES

Yaa Yeboaa Asare[1], Jennifer Morgan[1], Sandra Benin[1], Susana Debrah Apraku[1], Mabel Sarpong-Duah[1], Charles Quaye[2], Lydia Mosi[1]

[1]West African Center for Cell Biology of Infectious Disease (WACCBIP) - Department of Biochemistry, C, Accra, Ghana, [2]Noguchi Memorial Institute for Medical Research, Accra, Ghana

Buruli ulcer (BU) is a skin necrotizing disease caused by *Mycobacterium ulcerans* (MU), characterised by painless lesions and treated with antimycobacterial agents. However, the presence of secondary bacteria in these lesions may lead to pain, delay in wound healing and antimicrobial resistance-related treatment inefficacy. Culturing has been used to depict the microbial diversity of BU lesions which does not account for unculturable and slow-growing culturable bacteria. Culture-independent studies often have small sample sizes and inadequate controls which require additional work. This study overcomes this by using *16S* rRNA metagenomic sequencing on a larger sample size, controls from BU-endemic communities in Ghana and investigates the role these secondary bacteria play on antimicrobial resistance to the treatment regimen for BU. Samples were collected from 20 endemic communities in Ghana. BU confirmation was performed using *IS2404* qPCR. Metagenomic sequencing and culturing were used to establish the microbiome profile of the lesions and recover bacteria and determine their antimicrobial profiles to selected antibiotics that are the conventional and non-convential treatment drugs for BU. 67 samples were collected and 11 tested positive for BU. Sequencing identified a wider range of genera than from culturing. Alpha diversity analysis exposed no significant difference in bacterial diversity and evenness within BU positive and negative samples while beta diversity indicated statistically significant differences in community composition between the two groups. Almost all recovered bacteria were resistant to the selected antibiotics barring 10 % which were susceptible to Rifampicin and 5 % which showed intermediate susceptibility to Clarithromycin. This study has shown that there is varying bacterial diversity in BU and non-BU lesions, and normal skin commensals such as *S. aureus* can transition into opportunistic bacteria thereby causing pain and contributing to antimicrobial resistance-related treatment inefficacy. This knowledge is pivotal as it contributes to steps to review the current BU treatment regimens.

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CONFIRMING NONENDEMICITY AND CLOSING FINAL GAPS TOWARD ONCHOCERCIASIS ELIMINATION: OV16 SEROSURVEYS AND ONCHOCERCIASIS ELIMINATION MAPPING (OEM)-IMPACT ASSESSMENT EVIDENCE FROM BAYELSA AND RIVERS STATES, NIGERIA

Joseph Kwaghga Kumbur

CBM International, Abuja, Nigeria

Nigeria continues to accelerate progress toward onchocerciasis elimination through intensified epidemiological and entomological assessments. To clarify the endemicity status of Bayelsa and Rivers States—historically understudied due to their riverine ecologies—Onchocerciasis Elimination Mapping (OEM) and Impact Assessments were conducted using WHOrecommended OV16 serological methods. Dried blood spot samples were collected from likely blackfly breeding sites and analysed using the AP ELISA OV16 assay at the Molecular Epidemiology Laboratory, Osogbo. In Bayelsa State, 3,037 samples analysed during the Impact Assessment showed no OV16positive cases, while OEM testing of 763 individuals detected one positive in Ogbotobo community, Ekeremor LGA. In Rivers State, 6,096 individuals tested during the Impact Assessment were all negative, whereas OEM analysis of 5,435 samples identified two positives in Kpor community, Gokana LGA. Impact Assessment seroprevalence for both states was 0%, with upper confidence limits below the WHO <0.1% threshold for interruption of transmission. OEM findings revealed localized serological signals. Under WHO OEM 2024 guidelines, more than one positive in a single community meets the criterion for classifying an assessment unit as endemic, as seen in Gokana LGA. These isolated positives indicate the need for targeted community followup despite strong statelevel evidence of nonendemicity. Overall, the combined findings from both states provide robust epidemiological evidence supporting national decisionmaking toward onchocerciasis elimination verification, while highlighting the importance of continued monitoring to address localized transmission risks.

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LEVERAGING COMMUNITY HEALTH PROMOTERS TO INFLUENCE SOCIAL BEHAVIOR CHANGE IN CONTROL AND ELIMINATION OF SCHISTOSOMIASIS AND SOIL-TRANSMITTED HELMINTHS IN THE WESTERN REGION, KENYA

Paul M. Ndungu[1], Clare Amuyunzu[2], Mary K. Amuyunzu-Nyamongo[3], Rose Nkirote Mwirigi[2], Wyckliff Peter Omondi[4], Irene Natalie Chami[5], Rebecca Nyankeya[6]

[1]African Institute for Health and Development, Nairobi, Kenya, [2]Nairobi, Kenya, [3]AIHD, Naibobi, Kenya, [4]Ministry of Health, Nairobi, Kenya, [5]The END Fund, New York, NY, United States, [6]The END Fund, Nairobi, Kenya

Neglected Tropical Diseases (NTDs) continue to cause suffering, disability and deaths in Kenya. Schistosomiasis (SCH) and soil-transmitted helminths (STH) remain significant public health problems. Despite the two diseases being preventable infections, more than 6 million are infected while approximately 15 million are at risk of infection in Kenya. Community health promoters (CHPs) are the foundation of primary healthcare as they offer continuous preventive and promotive services. Kenya NTD program and County Governments with support from partners have tapped into CHPs in promoting social behavior change towards addressing SCH and STH in Western Kenya. The project identified and sensitized approximately 600 CHPs on SCH and STH. CHPs create awareness of the two diseases in target communities. They use local languages to create awareness and customize health messages to various groups within their communities.

They regularly leverage platforms such as public meetings, funerals, religious gatherings and peer meetings to disseminate information on prevention and control of the two diseases. Through annual assessments, the project has recorded improved outcomes in knowledge and adoption of preventive behavior. Knowledge levels on the two diseases have increased from 92.1% in 2024 to 93% in 2025 and 45% in 2024 to 58.7% in 2025 for STH and SCH, respectively. There has been remarkable increase in the proportion of people constructing and using toilets for human waste disposal at 95.1% and 98.2%, respectively in 2024 and 2025 respectively, while access to a functional handwashing facility at home improved to 55.1% in 2025 from 45.5% CHPs were cited as the most effective and preferred communication channel at 43.7% in 2024 and 51.8% in 2025. Community voluntarism, through CHPs in Kenya, is recognized for the pivotal role it plays in the prevention and control of disease. CHPs are critical change agents that provide effective and sustainable social behavior change in endemic communities.

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RELIGIOUS LEADERS AS CHANGE AGENTS IN ADDRESSING SCHISTOSOMIASIS AND SOIL TRANSMITTED HELMINTHIASIS IN KENYA

Rose N. Mwirigi[1], Clare Amuyunzu[2], Mary A. Nyamongo[2], Wyckliff Peter Omondi[1], Irene Natalie Chami[3], Rebecca Nyankieya[4], Paul Ndungu[2]

[1]Ministry of Health, Nairobi, Kenya, [2]African Institute for Health and Development, Nairobi, Kenya, [3]The END Fund, New York, NY, United States, [4]END Fund, Nairobi, Kenya

A high proportion of Kenyans report to belong to a religious faith 85.5% Christianity, 10.9% Islam, 1.5% no religion, 0.7% traditional faiths, and 1.3% others (Census data, 2019). Driven by the need to leave-no-one-behind in the control and elimination of schistosomiasis (SCH) and soil-transmitted helminthiasis (STH), the Ministry of Health Kenya, County Governments of Western Kenya and partners tapped into this resource in the implementation of a Social Behavior Change Communication (SBCC) project aimed at the control and elimination of SCH and STH. Engagement of religious leaders (both Muslim and Christian) involved the development of religious manuals that draw lessons from the Quran and Bible, respectively. The development of the two manuals brought together stakeholders from the MoH, Ministry of Education, religious leaders (Christians and Muslims), and partners. The participants identified information/verses that are aligned with prevention and control of STH and SCH from the Bible and Quran. The draft manuals were reviewed by groups of religious leaders from the four project counties and the feedback incorporated in the final revisions. The project has sensitized 102 religious leaders and equipped them with advocacy skills. The religious leaders have leveraged on their platforms to pass information on STH and SCH. Quarterly progress reports (2021-2025) indicate that the religious leaders have 716,954 community members (estimates drawn from the people they serve). About 49% of the respondents in the annual assessment (2024/2025) indicated that they had received information on STH/SCH from religious leaders (n=440/892). The religious leaders have influenced the construction and use of toilets/latrines (3430 households) and handwashing facilities in church compounds and at the community level (6,574). The involvement of religious leaders in health campaigns is made possible by the respect and trust they have with their large population of followers. They have ready platforms they can use to pass health information. We see religious leaders as a sustainable structure for SBCC at the community level.

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HEALTH SYSTEM READINESS FOR VISCERAL LEISHMANIASIS ELIMINATION IN KENYA: A MULTI-FACILITY ASSESSMENT ACROSS ENDEMIC COUNTIES

Robert Ofwete[1], Daniel Mwiti Ringera[2], Paul Kibati[2], Dollycate Njagi[3], Michael Ofire Ofire[2], Wyckliff Peter Omondi[4]

[1]Amref Health Africa, Nairobi, Kenya, [2]Nairobi, Kenya, [3]Dollycate Njagi, Nrb, Kenya, [4]Ministry of Health, Nairobi, Kenya

Kenya is accelerating efforts toward visceral leishmaniasis (VL) elimination; however, progress is constrained by uneven health system capacity across endemic counties. A cross-sectional health facility assessment conducted between November 2025 and March 2026 evaluated readiness for VL service delivery across multiple levels of care, focusing on infrastructure, diagnostics, human resources, referral systems, and availability of essential services. Facilities assessed were predominantly rural and serve har-to-reach populations. While most facilities had permanent structures, critical infrastructure gaps persist, with several requiring major renovation and upgrades. VL diagnostic capacity remained highly centralized at sub-county hospitals, with peripheral facilities dependent on referral systems that often involve long distances and weak transport linkages, contributing to delays in diagnosis and treatment. Lower-level facilities demonstrated minimal capacity for VL case management, characterized by limited availability of essential equipment, limited skillset among health care workers, suboptimal laboratory services, and lack of commodities for treatment. Human resources were disproportionately concentrated in higher-level facilities, leaving health centres and dispensaries understaffed and unable to effectively support early case detection and management. Although delivery of essential outpatient services was generally maintained, inpatient care capacity and comprehensive clinical management were limited outside referral facilities. System enablers, including reliable electricity, cold chain systems, water supply, communication, and transport, were inconsistently available and further constrained service delivery. These findings highlight critical programmatic gaps in decentralization, capacity building, and health system strengthening. Addressing these bottlenecks through targeted investments in diagnostics, workforce distribution, infrastructure, and referral systems will be essential to accelerate progress toward sustainable VL elimination in Kenya.

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QUANTIFYING HEALTH AND FOOD SECURITY RETURNS OF WHO PREVENTIVE CHEMOTHERAPY FOR SCHOOL-AGE CHILDREN ACROSS FOUR NEGLECTED TROPICAL DISEASES, 2010-2023

David Ipogah[1], Alexandra Sack[2], Jason R. Rohr[1]

[1]University of Notre Dame, Notre Dame, IN, United States, [2]The Carter Centre, Atlanta, GA, United States

Preventive chemotherapy for neglected tropical diseases generates substantial health and nutritional benefits, yet both dimensions have rarely been quantified together at global scale. We estimated health gains and food security value of WHO-coordinated preventive chemotherapy targeting ascariasis, trichuriasis, hookworm disease, and schistosomiasis among school-age children aged 5-14 years across 204 countries, 2010-2023. Infection intensity was inferred from Global Burden of Disease 2024 prevalence using species-specific negative-binomial distributions, partitioning children into intensity compartments calibrated to WHO morbidity thresholds. Efficacy priors were propagated under WHO observed, universal 50%, and universal 75% coverage scenarios against a no-treatment counterfactual. Disability-adjusted life years averted used intensity-stratified disability weights. Food security benefits were estimated using a nutrient-loss model converting tissue-mass deficit and blood-loss iron depletion to kilocalorie equivalents, with a trial-anchored sensitivity informed by the 0.15 kg weight gain estimate from Croke et al. (PNAS 2024), monetized using diet-weighted kilocalorie prices across 119 countries. Under WHO observed coverage, preventive chemotherapy averted an estimated 113 million disability-adjusted life years, representing

approximately 31% of the counterfactual burden, and preserved an estimated US$568 million in food value — equivalent to approximately 3.8 billion person-days of adequate nutrition. Health gains valued at one to three times gross domestic product per disability-adjusted life year yielded US$184-552 billion. Universal 75% coverage would avert approximately 211 million disability-adjusted life years and preserve US$966 million in food value. All directional sensitivity analyses confirmed conservative lower-bound estimates. To our knowledge, this is among the first studies to simultaneously quantify health and food security returns of preventive chemotherapy for school-age children globally, offering integrated evidence for WHO 2030 NTD Roadmap targets.

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FIFTEEN YEARS OF MASS ADMINISTRATION OF BENZIMIDAZOLES, BUT STILL PERSISTENT HIGH SOIL-TRANSMITTED HELMINTHIASIS IN RWANDA: ARE BENZIMIDAZOLES STILL EFFICACIOUS? WHAT FACTORS DRIVE THEIR EFFICACY STATUS?

Jean Bosco Mbonigaba

Rwanda Biomedical Center - University of Rwanda, Kigali/ Rwanda, Rwanda

Rwanda has implemented nationwide mass drug administration (MDA) for soil-transmitted helminths (STH) since 2008, achieving high coverage (>90%) and delivering >100 million treatments by 2023. However, the prevalence remains persistently high in almost half of the districts that were high endemic in 2008. There are growing concerns about reduced efficacy of benzimidazoles due to prolonged use, as well as increasing fears of resource loss from implementing MDA with potentially inefficacious benzimidazole monotherapy. We conducted a secondary analysis of albendazole data from an albendazole-ivermectin community therapeutic efficacy study (November 2023) in Rubavu District, Mudende Sector—an area with persistently high STH infections. Participants were stratified by cumulative MDA exposure (1-5, 6-10, ≥11 years) based on the history of MDA in the community. Logistic regression was used to assess associations between exposure duration and cure (categorical outcome), adjusting for baseline intensity categories, age, and sex. Log-transformed egg counts were used as a continuous outcome in sensitivity analyses alongside baseline intensity categories. Duration of exposure was not associated with cure across outcomes (all p>0.05). Baseline intensity strongly predicted cure. In categorical models, higher intensity reduced the odds of cure for any STH (aOR 0.56, 95% CI: 0.35-0.87; p=0.009) and Trichuris (aOR 0.12, 95% CI: 0.01-0.47; p<0.001), with a borderline association for Ascaris (p=0.071). In continuous models, EPG was associated with lower odds of cure for Ascaris (aOR 0.77, 95% CI: 0.58-0.98; p=0.030) and Trichuris (aOR 0.51, 95% CI: 0.41-0.63; p<0.001). Age was associated with Ascaris cure (p=0.023), while sex was not. There was no evidence of reduced albendazole efficacy with prolonged MDA exposure in this setting. Baseline infection intensity remains the key determinant of treatment response, supporting targeted strategies for high-intensity infections. Continued use in this setting, along with efficacy surveillance across settings with both high and low persistent prevalence, is recommended.

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DEVELOPMENT OF A COHORT STATE-TRANSITION MODEL TO ESTIMATE DISTRICT-LEVEL YELLOW FEVER VACCINATION COVERAGE IN GHANA

Mirava Yuson[1], Nicole Jenna Stephan[1], Sofia Machuca[1], Robert Ossom[2], Horlali Gudjinu[2], Pamela Quaye[3], Dennis Odai Laryea[2], Franklin Asiedu-Bekoe[2], Ernest Kenu[2], Seth Judson[1]

[1]Department of Medicine, David Geffen School of Medicine at UCLA, Los Angeles, CA, United States, [2]Disease Surveillance Department, Public Health Division, Ghana Health Service, Accra, Ghana, [3]Expanded Programme on Immunisation, Disease Control and Prevention Department, Public Health Division, Ghana Health Service, Accra, Ghana

Global vaccine shortages often result in limited access and unequal immunization coverage, hindering prevention and outbreak response to vaccine-preventable diseases such as yellow fever (YF). The Eliminate Yellow Fever Epidemics (EYE) strategy has targeted at least 50% immunization coverage for YF in high-risk African countries, such as Ghana, with a recommended threshold of 80% coverage to prevent epidemics. However, sub-national immunization coverage estimates have been challenging in the setting of changing demographics and varying immunization data. Therefore, we developed a district-level, age-structured cohort-state transition model to estimate YF immunization coverage from 2016-2025 across all 261 districts in Ghana.To calculate the proportion of population vaccinated against yellow fever per year in every district, we designed a cohort-state-transition model (cSTM) with two cohorts: infant (<1 year old) and non-infant (>1 year old) based on age-structured population estimates. Proportions from each cohort were transferred into either a vaccinated or unvaccinated state based on annual routine immunization or supplementary campaigns. The total number of vaccinated individuals were summed together with the previous year's vaccinated cohort population (assuming lifelong immunity) to calculate the cumulative vaccination coverage per year.We estimated that vaccination coverages in 2025 exceeded 50% in all districts and surpassed 80% in 146/261 (56%) of districts. Comparing our model estimates to regional-level Demographic and Health Survey data, we found a Mean Absolute Error (MAE) of 6%. Our findings highlight the heterogeneity of coverages between districts, and illustrate the model's potential use for identifying areas for targeted vaccination campaigns. Furthermore, our model is a versatile tool that can be adapted to other vaccine-preventable diseases in resource-limited countries.

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STRUCTURAL DRIVERS OF SOCIAL AND ECONOMIC EFFECTS OF TRACHOMATOUS TRICHIASIS (TT) AMONG WOMEN IN NORTH POKOT, KENYA: A FEMINIST POLITICAL ECONOMY ANALYSIS

Victoria Akoth Akoth

St. Paul's University, Kenya, Nairobi, Kenya

Trachomatous trichiasis (TT), the blinding sequelae of trachoma, is a disease of inequity. Global Health narratives have often reduced trachoma to a biomedical or behavioral issue. Nevertheless, a disproportionate burden exists among women in endemic regions like North Pokot. This study critically examined the structural drivers of social and economic effects of TT and implications for quality of life among women in North Pokot, Kenya. This study centered the lived experiences of women, within a critical qualitative paradigm. In-depth interviews were conducted among TT un-operated (12) and operated women (12). Purposive sampling ensured representation across geography, age and TT severity. Key Informant interviews (10), Focus Group Discussions (four) among: Spouses of operated, Spouses of un-operated women, Women who never had TT and Community Health Promoters provided triangulation and contextualizing individual narratives within broader historical and institutional frameworks. Data analysis, utilizing intersectional and thematic analysis interpreted how systems political, economic, and social shape the lived experiences with TT. Social and economic devastation due to TT including TT recurrence, was exacerbated by intersecting systems of oppression directly linked to neglect and under-investment in water, sanitation, and healthcare infrastructure. Additionally, gendered power dynamics within restrictive social norms restricted women's autonomy in seeking TT surgery, systematically delaying surgery until irreversible blindness. Majority of the women felt that their normal participation in economic and social activities was diminished hence asset depletion, dependence, social stigma and inability to fill gendered roles. The surgery delays were not an individual choice, but signify structural violence characterized by infrastructural neglect. Achieving health equity requires moving beyond surgical campaigns to embrace policy reforms targeting root causes of marginalization towards reaching the last mile to eliminate trachoma and preventive recurrence.

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LIVESTOCK AS SENTINELS: MULTIPLEX DETECTION OF ZOONOTIC VIRAL EXPOSURE IN GHANA

Jennifer Nyamekye Yanney[1], Patrick Kwasi Obuam[2], Millie-Cindy Aba Aude Koffi[1], Keersten Michelle Ricks[3], Millicent Owusu[1], Shirley Nimo-Paintsil[4], Seth Offei Addo[1]

[1]Noguchi Memorial Institute for Medical Research, Accra, Ghana, [2]Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, [3]United States Army Medical Research Institute of Infectious Diseases, Frederick, MD, United States, [4]U.S. Naval Medical Research Unit EURAFCENT, Ghana Detachment, Accra, Ghana

Livestock play a vital role in rural livelihoods and food security, especially in Sub-Saharan Africa, where they may serve as reservoirs or amplifying hosts for zoonotic viruses. Despite this, monitoring for viral exposure in animal populations remains inadequate in many endemic regions. This study aimed to assess serological evidence of virus exposure in livestock using a high-throughput multiplex platform. The Magpix (Luminex-based) multiplex assay was employed to screen dry blood spots (DBS) for antibodies against a panel of viral pathogens, enabling the simultaneous detection of multiple targets from a small sample volume. This approach is particularly suitable for field investigations where sample storage and transportation may be limited. A total of 417 DBS were collected from goats (38.6%), sheep (33.1%), and cattle (28.3%) across the Upper East (60.9%), Ashanti (30%), and Bono East (9.1%) regions. The livestock were predominantly female (71.9%) and under one year of age (60.7%). The seroprevalence rates recorded included West Nile (36%), Dengue (34.8%), Crimean-Congo Haemorrhagic Fever Virus (29.7%), Chikungunya (17.5%), Rift Valley Fever (6.7%), Tick-Borne Encephalitis (6.5%), Lassa (2.4%), and Nipah (1.2%). It was observed that 17.0% of the livestock were exposed to a single virus, 18.0% to two distinct viruses, 13.7% to three viruses, 6.0% to four viruses, and 3.4% to five viruses. Overall, a significant association was identified between pathogen exposure and demographic variables such as host, sex, region, and age group ($p<0.001$). This study provides important baseline data on viral exposure in Ghanaian livestock and demonstrates the effectiveness of multiplex serology for One Health surveillance. Strengthening these techniques is essential for improving outbreak preparedness and guiding targeted measures to reduce zoonotic disease risks.

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POST-MISSION METAGENOMIC SURVEILLANCE OF BLOOD MICROBIOTA IN MILITARY WORKING DOGS

Oleksandra Kepple[1], Le Jiang[2], Jennifer Safko[3], Hsiao-Mei Liao[4], Fernanda Fortes de Araujo[5], Robert Fathke[6], Naomi E. Aronson[7]

[1]HJF-USUHS, Bethesda, MD, United States, [2]Naval Medical Research Command, Silver Spring, MD, United States, [3]Univ of Georgia, Athens, GA, United States, [4]Silver Spring, MD, United States, [5]USUHS, Bethesda, United States, [6]San Antonio, TX, United States, [7]USUHS, Bethesda, MD, United States

Military working dogs (MWDs) play an important role in combat and Special Operations. During deployment MWDs live closely with their handlers. In conflict zones MWDs may provide valuable insights into zoonotic pathogens and antimicrobial resistance, informing about environmental threats to the operational readiness of dog and human warfighters. We used metagenomic next-generation sequencing to characterize blood microbiome of MWDs post-deployment to Iraq. DNA from banked whole blood collected within 6 months of return from 50 MWDs was extracted using a QiAamp blood mini kit. After host DNA depletion and library preparation, samples were multiplexed and sequenced on a nanopore sequencer. Data were analyzed via the Chan Zuckerberg ID pipeline for unbiased taxonomic identification, followed by NCBI BLAST verification. Metadata (medical history and diagnoses) were abstracted from the records and correlated with pathogen detection and abundance. We observed potentially antimicrobial-resistant (AMR) pathogens in the blood: *Acinetobacter baumannii* (the "Iraqibacter"), *Stenotrophomonas maltophilia* and *Pseudomonas aeruginosa* in both symptomatic (57%) and asymptomatic (43%) groups. One dog tested positively for 2 *Shigella* species (2%). Three (6%) dogs had diarrhea during or after deployment. Previous antibiotic use was noted in 11/16 (68%) MWD with AMR bacteremia vs 11/34 (32%) who had no AMR bacteremia. Clinical laboratory values were similar in those with and without bacteremia. In conclusion, Our data provided a single time point of blood microbiome in MWDs post-deployment to Iraq. Identified bacterial species can be linked to MWD's environmental exposures and intense physical exertion possibly associated with certain disease conditions such as diarrhea or AMR bacteremia. Additionally, MWDs could serve as a sentinel and reservoir for human infections through the uniquely close relationships developed during combat.

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RECOMBINANT *LACTOBACILLUS ACIDOPHILUS* AS AN AVIAN VACCINE AGAINST WEST NILE VIRUS

Jordyn Walker

Jordyn Walker, Fort Collins, CO, United States

West Nile virus (WNV) is a neuroinvasive flavivirus which first emerged in the United States in 1999. Since its emergence, WNV has become the most widespread arbovirus in the country, causing approximately 50,000 West Nile disease cases and nearly 3,000 deaths. Currently, there are no human treatments or vaccines for WNV. Effective vaccines have been developed for horses; however, horses are incidental hosts and therefore are not implicated in the WNV transmission cycle. Insecticides are the main form of WNV transmission control and are sprayed as adulticides or used as larvicides. These can reduce mosquito population numbers, but they are often used reactively, so their ability to reduce WNV transmission is low, and their efficacy is further reduced by resistance in the mosquitoes, cost and negative public perceptions. As a result of these issues with current control methods, the need for novel and effective control strategies for WNV is crucial. The transmission cycle of WNV is maintained between *Culex* mosquitoes and birds, but there are no approaches to WNV control that target birds. We hypothesize that orally immunizing birds using recombinant *Lactobacillus acidophilus* (rLa) expressing antigenic WNV proteins could reduce WNV transmission between birds and mosquitoes, thereby protecting both birds and human populations. We have generated live rLa expressing the WNV proteins premembrane (prM) and non-structural protein-1 (NS1) and are currently working to produce rLa expressing envelope (E) protein. These constructs are additionally modified to express adjuvant proteins. Testing so far indicates these probiotic vaccines are safe in different bird species, easy to produce, and induce antigen specific immunity, making this approach a potentially viable option for the control of WNV transmission using a One Health approach.

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FROM ANIMAL SEROPREVALENCE TO HUMAN SPILLOVER: ECO-EPIDEMIOLOGICAL MODELLING OF CRIMEAN-CONGO HEMORRHAGIC FEVER VIRUS IN RURAL TURKEY

Caitlin Cotter, Gabrielle Kostecki, Dennis Axel Bente

University of Texas Medical Branch, Galveston, TX, United States

Crimean-Congo hemorrhagic fever virus (CCHFV) circulates in a complex eco-epidemiologic system, mainly involving *Hyalomma spp*. ticks as vector-reservoirs, small mammals (rodents, lagomorphs) and domestic ruminants (cattle, sheep, goats, camels) as amplifying hosts for the ticks, and humans as incidental spillover hosts. Numerous reports documenting high levels of clinically inapparent seropositivity in livestock have highlighted a substantial risk to exposed humans, yet translating animal serology into human infection risk remains challenging. We developed a two-stage transmission framework relevant to pastoral systems in Turkey: a deterministic age-structured SIRS model for livestock and a stochastic SEIRS model for spillover to humans, with force of infection driven by infectious livestock prevalence, cattle density, and environmental determinants of tick abundance. Assuming relatively stable CCHFV prevalence in ticks and short, low-level livestock viremia, we explored how cattle density, tick

activity, waning livestock immunity, and choice of husbandry practices could shape transmission and occupation-specific human risk. By systematically probing structural and parametric uncertainties in the model, we identified critical data gaps, including the duration and effectiveness of post-infection immunity in cattle, which will inform prioritization of field observational studies and experimental studies to be conducted in new, ABSL-4 livestock facilities. Model predictions and validation will support targeted prevention and control strategies for rural populations in Turkey and other CCHFV-endemic regions.

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ASSOCIATIONS BETWEEN SEASON, WEATHER, AND ABUNDANCE OF EXTENDED-SPECTRUM BETA-LACTAMASE-PRODUCING E. COLI (ESBL-EC) IN HOUSEHOLD HUMAN, ANIMAL, AND ENVIRONMENTAL RESERVOIRS IN RURAL BANGLADESH

Gabriella Rose Barratt Heitmann[1], Md. Sakib Hossain[2], Sumaiya Tazin[3], Claire Anderson[4], Suhi Hanif[5], Farjana Jahan[6], Abul Kasham MD. Shoab[7], Zahid Hayat Mahmud[8], Md Mahbubur Rahman[9], Ayse Ercumen Ercumen[10], Jade Benjamin-Chung[4]

[1]Stanford University, Palo Alto, CA, United States, [2]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [3]Raleigh, NC, United States, [4]Stanford University, Stanford, CA, United States, [5]Palo Alto, CA, United States, [6]International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Dhaka, Bangladesh, [7]DHAKA, 13, Bangladesh, [8]ICDDRB, Dhaka, Bangladesh, [9]Intl Ctr for Diarrhoeal Dis Res, Bangladesh (icddr,b), Dhaka, 13, Bangladesh, [10]North Carolina State Univ, Raleigh, NC, United States

Studies linking weather and antimicrobial resistance have overlooked how weather and season affect AMR dynamics in household human, animal, and environmental reservoirs. We estimated associations between weather, season, and extended-spectrum beta-lactamase-producing E. coli (ESBL-Ec) abundance in rural Bangladesh. In 112 households from the CRADLE trial, we enumerated ESBL-Ec using CHROMagar ESBL across floor swabs, courtyard soil, chicken and cow feces and child stool. Daily weather variables (mean and maximum temperature and relative humidity [RH], total precipitation) were pulled from ERA5 Land Hourly for 1, 2, 3 and 7 days prior to sample collection and matched to household geocoordinates. Generalized linear models for log10 ESBL-Ec colony-forming units were stratified by sample type and adjusted for trial arm and wealth quintile. Associations with temperature in courtyard soil varied qualitatively by season: positive in winter (1-day lag: 0.36 [0.15, 0.56] per 1°C) but negative (7-day lag: –0.35 [–0.71, 0.00]) in monsoon; there were positive associations with RH (7-day lag: 0.10 [0.01, 0.19]) and negative associations with precipitation (–0.22 [–0.36, –0.07] per 100mm) across seasons. Associations in chicken feces were consistent across seasons but strongest in the dry season: negative with temperature (–0.66 [–1.18, –0.13] per 1°C) and positive with RH (0.53 [0.18, 0.88] per 1% max RH). Associations in floor swabs were positive with RH in the dry season (0.04 [0.00, 0.09]) and negative with temperature in the monsoon (–0.38 [–0.76, 0.00]). Child stool was negatively associated with winter temperature (–0.25 [–0.50, –0.01]). No weather associations were found for cow feces. Except for courtyard soil, temperature was predominantly negatively associated with ESBL-Ec across reservoirs; RH was predominantly positive. These results highlight seasonal and reservoir variation in household ESBL-Ec abundance, complicating simple positive temperature associations reported in clinical surveillance data. Potential interventions should consider how season and weather influence outcomes in relevant reservoirs.

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MICROBIAL DIVERSITY AND PATHOGENS OF MEDICAL IMPORTANCE ASSOCIATED WITH *PERIPLANETA AMERICANA* IN URBAN PANAMA

Ariel H. Magallon-Tejada[1], Steven Gonzalez Del Cid[1], Oriel Lezcano[1], Kharim Hernández[2], Cibeles Gaitan[2], Cely Gonzalez[1], Ríos-Sanjur Katia[1], Alexander Martínez[1], Rina Sobarzo[2]

[1]Instituto Conmemorativo Gorgas de Estudios de la Salud, Panama, Panama, [2]Universidad Autónoma de Chiriquí, Chiriquí, Panama

Domestic cockroach *Periplaneta americana* represents a critical interface linking environmental contamination, animal reservoirs, and human health in urban ecosystems. This synanthropic species is widely distributed and continuously exposed to diverse microbial communities. Its omnivorous feeding and high mobility enable both mechanical transmission of pathogens on its body surface and biological carriage within its digestive tract, facilitating dissemination across environmental and human interfaces. This dual role as a mechanical and biological vector underscores its epidemiological importance and highlights the need for integrated surveillance and control strategies. We assessed clinically relevant parasites, yeasts, and bacteria associated with *P. americana* collected from urban areas, including hospital surroundings. A total of 750 specimens were analyzed using optical microscopy, conventional PCR, selective culture media, VITEK2, MALDI-TOF MS, and next-generation sequencing (NGS). Protozoan trophozoites identified included *Entamoeba* sp., *Cryptosporidium* sp., *Eimeria* sp., and *Lophomonas blattarum*, along with helminth eggs, cysts, and larvae of *Strongyloides*, *Uncinaria*, and *Taenia*, predominantly recovered from the digestive tract. Clinically relevant yeasts included *Candida albicans*, *C. glabrata*, *C. tropicalis*, *C. krusei*, and *C. parapsilosis*, as well as *Trichosporon asahii* and *T. inkin*, with several isolates showing resistance to fluconazole, voriconazole, caspofungin, amphotericin B, and 5-fluorocytosine. Bacterial isolates of major public health significance included *Klebsiella pneumoniae*, *Acinetobacter baumannii* complex, *Escherichia coli*, *Enterococcus faecium*, *Serratia marcescens*, *Stenotrophomonas maltophilia*, and *Staphylococcus aureus*. These findings confirm *P. americana* as a key mechanical and biological vector in urban and hospital environments, highlighting the urgent need for integrated pest management and public health surveillance to mitigate the risk of infectious disease transmission in human populations.

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A ONE HEALTH APPROACH TO INVESTIGATE SUSTAINED SOIL TRANSMITTED HELMINTH TRANSMISSION IN RURAL SETTINGS OF MEGHALAYA, INDIA

Uniqueky Gratis Mawrie

London School of Hygiene and Tropical Medicine, London, India

Soil-transmitted helminths (STH) remain a significant public health concern in low- and middle-income countries, despite mass drug administration. While reductions in high-intensity infections have been achieved, low-prevalence and low-intensity infections persist. Zoonotic STH species such as *Ancylostoma ceylanicum*, *A. caninum*, *A. braziliense*, *Ascaris suum*, and *Trichuris vulpis* may contribute to transmission, but data remain limited. This study adopts a One Health approach to investigate prevalence and distribution of STH species across humans, animals, and environmental reservoirs. A cross-sectional study was conducted in tribal settings of Meghalaya, India, involving 1,951 humans from 444 households and 340 animals. Stool samples from humans and animals, and soil samples from households and common areas, were tested using species-specific quantitative polymerase chain reaction. Prevalence with 95% confidence intervals was estimated using survey-weighted methods and Taylor linearization. Individual and household-level risk factors were assessed using multilevel logistic regression, defining “wormy households” as those with concurrent STH positivity in humans, animals, or soil, or multiple infections. Overall prevalence of STH in humans was 20.3% (95% CI: 5.8–25.7); *N. americanus* (14.9%) was the most common species, followed by *A. ceylanicum* (4.4%). Among animals, prevalence was highest in pigs

(45.1%; 95% CI: 39.1–51.2), followed by dogs (41.9%; 95% CI: 27.3–58.1) and goats. About 76% (95% CI: 69.7–80.8) of household soil samples were positive, mainly with *A. ceylanicum*. At the household level, 58.6% had at least one infected individual, and 13.1% had concurrent positivity in humans, animals, and soil. Increasing age (AOR 11.6; 95% CI: 4.9–27.6) and male gender (AOR 1.8; 95% CI: 1.4–2.3) were associated with higher odds of infection. Tribal (OR 2.2; 95% CI: 1.2–4.3) and farming households (OR 1.9; 95% CI: 1.2–3.0) had higher odds of being a "wormy household". These findings highlight persistent STH transmission, including zoonotic species, and underscore the need for integrated One Health approaches for control.

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IMPLEMENTATION OF COLLABORATIVE EFFORTS ACROSS THE SOUTHCOM NETWORK FOR RESEARCH AND SURVEILLANCE IN ONE HEALTH RED AMERICANA DE INVESTIGACIÓN Y MONITOREO - UNA SALUD RAIMUS

Samantha Michelle López[1], Andrea E. Arcos Villalva[1], Madeline Kernahan[2], Abigale Proctor[3], Christopher N. Mores[4], Maria E. Barnett de Antinori[5]

[1]The George Washington University, Washington, DC, United States, [2]Arlington, VA, United States, [3]Alexandria, VA, United States, [4]George Washington University, Washington, DC, United States, [5]Gorgas Memorial Institute, Panama City, Panama

Population growth, migration, geopolitical instability, and climate change continue to reshape human-environment interactions and the transmission dynamics of zoonotic pathogens. This global health challenge must be addressed through a coordinated One Health (OH) approach that integrates the human, animal, environmental, and policy sectors. The SOUTHCOM Red Americana de Investigación Monitoreo Una Salud (RAIMUS) was established to strengthen and harmonize infectious disease surveillance and response across Latin America and the Caribbean through regional collaboration. Through an online survey and virtual working group (WG) meetings, we collated environmental, animal, and human health concerns related to high-priority pathogens, regional OH perceptions, and existing surveillance capacity. Qualitative analysis and discussion identified variability in surveillance systems, limited cross-sector integration, and gaps in training and education. Building on these findings, RAIMUS established thematic WGs to operationalize the network's activities: two pathogen-focused groups to address avian influenza and Oropouche virus as models, alongside two cross-cutting groups, each focused on environmental health and governance and capacity strengthening. Through a series of virtual WG meetings and ongoing discussions via collaborative platforms, participants identified country-specific gaps, knowledge needs, ongoing response efforts, and proposed intervention strategies. These efforts led to an in-person regional meeting in Panamá, where stakeholders reviewed preliminary intervention concepts, exchanged perspectives across sectors, and refined proposal strategies, focusing on strengthening surveillance systems, enhancing cross-sector coordination, and strengthening capacities, with an emphasis on adaptable, regionally relevant approaches. Through fostering sustained partnerships among stakeholders, RAIMUS aims to improve early detection of emerging zoonotic threats and support coordinated, evidence-based responses, ultimately strengthening regional health security.

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EMERGENCE OF ZOONOTIC ENTEROTOXIGENIC MULTIDRUG-RESISTANT BACTERIA ISOLATED FROM *OREOCHROMIS MOSSAMBICUS* (BLACK TILAPIA) IN FISH FARMS AT MAFÉRÉ, ABOISSO, CÔTE D'IVOIRE

Bertin Kipre Guede[1], Valérie C. Gbonon[1], Victoire Gadou[2], Fernique K. Kouadio[1], Anatole A. Toty[1], Félicité Beudjé[1], Nathalie A. Guessennd[1]

[1]Institut Pasteur cote d'Ivoire, Abidjan, Côte D'Ivoire, [2]Université Péléforo Gon, Korhogo, Côte D'Ivoire

Oreochromis mossambicus is easy to raise and harvest; therefore, crossbred with other tilapia species in aquaculture. This fish represents around 4% of the total aquaculture productionworldwide. Yet, Breeders use antibiotics to primarily control low respiratory infections.Unfortunately, lack of stewardship, hygiene, and good fish farming practices often cause epidemic diarrheic illnesses, food poisoning, and contribute to bacterial resistance. In Côted'Ivoire, AMR prevalence is critically rising in agri-food sector. Current study in that vein aimsat detecting multidrug-resistant bacteria from fish farming ponds. Twelve fish species were collected over 3 weeks' time, then analyzed. For Salmonella spp, bufferedpeptone broth (BPB) was used for pre-enrichment before analysis from SS agar. For *V.cholerae*, peptone alkaline water enriched sample prior to analysis from TCBS Agar. As to *E.coli*, enrichment was carried out using buffered peptone broth, and EMB agar used for analysis. Microscopy allowed to identify Salmonella spp on SS agar, *V. cholerae* on TCBS, and *E. coli*on EMB prior to confirmation. Susceptibility tests showed resistance to Ceftriaxone,Chloramphenicol, and Nalidixic acid for Salmonella spp with respective prevalence rate of16.28%, 16.28%; Erythromycin and Clindamycin for *Vibrio cholerae* at 66% and 66%, and*Escherichia coli* for Tetracycline at 100%. Results showed fish farm products to harbor multidrug-resistant bacteria. This confirms previous studies on active circulation of bacterial communities in fish farm ponds carryingresistant plasmids. In conclusion, Data showed fish consumers to be exposed to multidrug resistant bacteria due touncontrolled use of antibiotics in aquaculture practices.

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PERSISTENT TRIPLE-CLASS CO-RESISTANCE IN CANINE URINARY *STAPHYLOCOCCUS PSEUDINTERMEDIUS*: A ONE HEALTH SURVEILLANCE SIGNAL AT THE ANIMAL-HUMAN INTERFACE

Chandika Damesh Gamage[1], Kamala Manda[1], Cam Lucas[1], Christine Walsh[1], Udeni B. R. Balasuriya[2], Alma Roy[1], Maria Mitchell[1]

[1]Louisiana Animal Disease Diagnostic Laboratory (LSU Diagnostics), Baton Rouge, LA, United States, [2]School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA, United States

Staphylococcus pseudintermedius is an important companion-animal pathogen and antimicrobial resistance concern at the animal-human interface, with direct relevance to stewardship and One Health surveillance. We retrospectively analyzed submission-based canine urinary *S. pseudintermedius* isolate entries with concurrent oxacillin, clindamycin, and enrofloxacin susceptibility results from a U.S. veterinary diagnostic laboratory from January 1, 2017, through December 31, 2025. Among 188 isolate entries, resistance was 43.6% for oxacillin, 39.4% for clindamycin, and 37.2% for enrofloxacin; enrofloxacin non-susceptibility reached 54.8%. The most common phenotypes were fully susceptible (SSS, 38.8%) and triple-resistant (RRR, 28.7%). Compared with oxacillin-susceptible isolates, oxacillin-resistant isolates were far more likely to be clindamycin-resistant (78.0% vs 9.4%), enrofloxacin-resistant (73.2% vs 9.4%), and resistant to both clindamycin and enrofloxacin (65.9% vs 5.7%; all $p<0.0001$). Annual oxacillin resistance remained present throughout the surveillance period and did not differ significantly by year ($p=0.483$). These findings identify a clinically important co-resistant urinary *S. pseudintermedius* population in dogs and support veterinary diagnostic laboratory data as a practical

sentinel for companion-animal antimicrobial resistance surveillance and stewardship. Integrated human-animal-environment studies are warranted to determine whether shared methicillin-resistance determinants contribute to MRSP-MRSA interchange at the companion animal-human interface.

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GEOSPATIAL AND SOCIO-ECOLOGICAL MODELING OF NIPAH VIRUS SPILLOVER RISK IN BANGLADESH

Rachel Erickson[1], Sazeda Akter[1], Lanszki Zsofia[2], Nurul Islam[1], Gabor Kemenesi[2], John Uelmen[1]

[1]University of Wisconsin-Madison, Madison, WI, United States, [2]University of Pecs, Pecs, Hungary

Nipah virus (NiV) is a highly pathogenic zoonotic paramyxovirus responsible for recurrent outbreaks of severe encephalitis in Bangladesh, where spillover events occur nearly every year, and the case-fatality rate exceeds 70%. The primary reservoir host, *Pteropus medius*, frequently roosts near human settlements and contaminates raw date palm sap. This exposure route is the primary mechanism of spillover. Environmental change, including deforestation and forest fragmentation, has increased bat reliance on cultivated food resources and promoted roosting in human-dominated landscapes. Despite these known drivers of spillover, gaps remain in identifying high-risk locations before outbreaks occur. This project aims to develop a predictive model of *P. medius* ecology as a key piece in understanding Nipah virus spillover risk in Bangladesh. First, we will develop and validate a geospatial model of bat roost locations and spillover risk using ArcGIS by integrating environmental, ecological, and anthropogenic data from fourteen known *P. medius* roost sites. Spatial variables will include land use, forest fragmentation, proximity to human settlements, vegetation cover, climate variables, and date palm tree distribution. Field surveys will characterize roost ecology and surrounding habitat and assess bat population size. To further refine and validate ecological predictions, a subset of bats will be fitted with GPS tracking devices equipped with tri-axial accelerometers to quantify movement patterns, foraging behavior, and interactions with date palm trees. Second, mobile-based knowledge, attitudes, and practices surveys will assess human behaviors related to date palm sap harvesting and consumption, bat interactions, and community awareness of Nipah virus risk. Geocoded survey data will be integrated with ecological and environmental layers to identify socio-ecological determinants of spillover. Model outputs will generate predictive risk maps that highlight areas of elevated spillover potential to inform targeted surveillance, prevention strategies, and early detection efforts.

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ONE HEALTH SURVEILLANCE OF *ANGIOSTRONGYLUS CANTONENSIS* IN GASTROPOD HOSTS IN SOUTHERNCALIFORNIA

Young Ji Kim

Loma Linda University Internal Medicine, LOMA LINDA, CA, United States

Angiostrongyliasis is aparasitic disease caused by *Angiostrongylus cantonensis*, a zoonotic nematode historically associated with tropical and subtropical regions but increasingly reported in new geographic areas. The presence of *A.cantonensis* in Southern California remains uncertain, despite the widespread distribution of potential gastropod intermediate hosts and suitable environmental conditions for transmission. In particular, it is unclear whether commonly encountered terrestrial and freshwater snails—including garden snails, snails within the tribe Thebini, and invasive apple snails (*Pomacea canaliculata*)—harbor this pathogen and contribute to potential human exposure.The presence of *A.cantonensis* in these gastropod populations would be of public health concern given therisk of contamination of produce and freshwater sources, as well as the potential for emergence of angiostrongyliasis in a non-endemic region. To assess this risk, we conducted a field survey of suspected intermediate hosts across urban, agricultural, and freshwater environments in Southern California, with subsequent laboratory analysis using microbiologic and molecular methods.Thus far, we have collected and processed a total of 13 gastropod specimens, including garden snails, members of the tribe Thebini, and apple snails. Processing using hydrochloric acid–pepsin digestion and light microscopy has not identified nematode larvae in any specimens to date. Molecular testing using qPCR for *A.cantonensis* is ongoing.Our preliminary findings have not demonstrated evidence of *A.cantonensis* in sampled gastropods; however, given the limited sample size, continued surveillance is warranted. Further sampling and molecular confirmation will be essential to determine whether *A.cantonensis* is present in Southern California and to better define the risk of angiostrongyliasis in this region.

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INCORPORATING SOCIODEMOGRAPHIC HETEROGENEITY INTO TRANSMISSION MODELS FOR RESPIRATORY DISEASES

Soren L. Larsen[1], Junke Yang[2], Erin Haslett[3], Alyssa Anastasi[4], Lydia Schieleit[5], Pamela P. Martinez[6], Ayesha S. Mahmud[1]

[1]University of California, Berkeley, Berkeley, CA, United States, [2]The Ohio State University, Columbus, OH, United States, [3]Emory University, Atlanta, GA, United States, [4]UW Madison, Madison, WI, United States, [5]University of Illinois, Urbana-Champaign, Urbana, IL, United States, [6]University of Illinois, Urbana Champaign, Urbana, IL, United States

Infectious diseases exhibit complex dynamics which are shaped by both biological and social factors. In the context of human respiratory pathogens, structural factors such as housing conditions or mobility patterns have been shown to drive different transmission risks across sociodemographic groups. Yet, social heterogeneity remains underexplored as a fundamental cause of respiratory infectious disease. To better understand the social and behavioral factors driving ecological dynamics of human respiratory pathogens, in 2024-2025, we fielded a statewide survey in Illinois to capture health status, health-seeking behaviors, and motivations across sociodemographic groups. We first analyzed vaccination across age, finding adults are more likely to be vaccinated for SARS-CoV-2 than influenza, while the reverse is true for their children. When analyzing reasons for vaccination behavior, over 70% of vaccinated respondents in each age bracket cited protecting themselves or others. Conversely, unvaccinated individuals reported highly heterogeneous motivations such as safety concerns, skepticism, or resource constraints, with no single motivation shared by the majority of respondents in any age group. Therefore, campaigns to increase vaccination coverage must be targeted to populations with distinct concerns. Second, across households with children, 19% of SARS-CoV-2 vaccinated adults live with an unvaccinated child, and 5% of vaccinated children live with an unvaccinated adult. We implemented transmission models to explore indirect effects of excluding children and young adults from vaccine eligibility, finding that associated absolute increases in deaths would be greatest for those over 65. These findings highlight the crucial role of considering sociodemographic heterogeneity in disease dynamics.

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FROM WASTING TO DIAGNOSIS: QUANTIFYING TUBERCULOSIS DIAGNOSTIC DELAYS IN THE BRONX POPULATION

Ayesha H. Sundaram[1], Yara Ahmed[2], Xianhong Xie[2], Tao Wang[2], Erika Orner[1], Phyu Thwe[1], Johanna Patricia Daily[2]

[1]Montefiore Medical Center, Bronx, NY, United States, [2]Albert Einstein College of Medicine, Bronx, NY, United States

New York City has a tuberculosis (TB) incidence rate of 7.8 per 100,000 persons, higher than the U.S. national average. Despite improved diagnostics, the total time to diagnosis varies significantly, and there is insufficient domestic research on contributing factors to delay. Using a retrospective design, we quantified the time to diagnosis in 125 patients with laboratory-confirmed TB in the Bronx from 8/2018-8/2025. In a multivariate analysis, we evaluated the association of demographic, socioeconomic and clinical variables with three time intervals: symptom onset to initial healthcare contact (OC), healthcare contact to diagnosis (CD), and total time to diagnosis (TT). Significance was defined in part by $p < 0.05$. Our study cohort was 58% male, 67% between ages 18-64, and

72% immigrants with most immigrating remotely (>5 years prior). Under half the cohort was privately insured with even smaller proportions of concurrent substance use and immunocompromise. Initial provider contact was the emergency department (72%) followed by medical subspecialty offices (13%) and primary care clinics (5.8%). Thirty-three percent of patients had extrapulmonary TB, surpassing the estimated national prevalence of twenty percent. Median TT was 57.5 days (IQR 23-124), OC 14 days (IQR 5-60) and CD 22 days (IQR 4-59.8). Patients with tobacco use and private insurance had longer OC. On multivariable analysis, extrapulmonary TB and TB-endemic country of origin were independently associated with longer CD. Extrapulmonary TB presentation, private insurance status, and greater number of pre-diagnostic visits (Spearman $\rho = 0.58$, $p < 0.0001$) related to longer TT; each additional visit contributed 23 days to TT. These findings highlight healthcare system and patient-level factors associated with prolonged time to diagnosis and identify opportunities to improve early recognition and care pathways.

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FUNCTIONAL CHARACTERIZATION OF METALLOCHAPERONE CANDIDATES RV0944 AND RV2102 IN THE ACTIVATION OF PYRAZINAMIDASE AND PYRAZINAMIDE SUSCEPTIBILITY IN *MYCOBACTERIUM TUBERCULOSIS*

Fresie Maciel Carpio, Mishell Landa Flores, Alonso Flores Bacayan, Sylvana Chirito Apolaya, Mirko Zimic, Patricia Sheen
Universidad Peruana Cayetano Heredia, Lima, Peru

Pyrazinamide (PZA) is a first-line antitubercular agent essential for treatment shortening because of its sterilizing activity against persistent *Mycobacterium tuberculosis* (Mtb) subpopulations. Its antimycobacterial effect depends on bioactivation by pyrazinamidase/nicotinamidase (PZase), a metalloenzyme encoded by *pncA*. Although *pncA* mutations are the main determinant of PZA resistance, impaired PZase metalation may also compromise drug bioactivation and could explain resistance in isolates retaining wild-type *pncA*. We hypothesized that Mtb uses metallochaperones to facilitate metal cofactor insertion into PZase and that loss of this function reduces conversion of PZA to pyrazinoic acid (POA), promoting a resistant phenotype. This study evaluated the role of *rv2102* and *rv0944*, two candidate PZase metallochaperones, in PZA action and resistance. Candidates were prioritized by bioinformatic functional and structural annotation, homology-based analyses, and molecular docking with PZase. Deletion mutants of *rv0944* and *rv2102* were generated in the H37Rv background using ORBIT recombineering. Functional contribution was assessed by POA efflux assays and PZA susceptibility testing in Mtb. In parallel, recombinant *rv0944* and *rv2102* were expressed in *E. coli* to evaluate in vitro restoration of metal-depleted PZase activity. H37Rv:Δ*rv2102* showed a significant reduction in POA efflux rate from days 0-10 compared with wild-type H37Rv (p=0.0018). However, linear regression analysis did not show complete abolition of POA production, in contrast to H37Rv:Δ*pncA*. This phenotype correlated with a partial increase in PZA MIC, with a twofold shift in H37Rv:Δ*rv2102.* In vitro reactivation assays showed that 5 μM recombinant *rv2102* restored 80% of the activity of 3 μM metal-depleted PZase (p<0.0001). These findings support a role for *rv2102* in PZase metalation, likely by facilitating Zn2+ incorporation, and provide mechanistic insight into unexplained PZA resistance and potential adjunct therapeutic targets in drug-resistant Mtb.

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DIAGNOSTIC PERFORMANCE OF MODIFIED FLUORESCENT ZIEHL-NEELSEN PROTOCOLS FOR TUBERCULOSIS DETECTION

Mishell Landa Flores, Fresie Denisse Maciel Carpio, Angel Agapito, Armando Flórez Troncos, Andrew Apolaya Leandro, Patricia Sheen Cortavarria, Mirko Zimic Peralta
Universidad Peruana Cayetano Heredia, Lima, Peru

Tuberculosis diagnosis in resource-limited settings still relies heavily on smear microscopy, but conventional Ziehl-Neelsen (ZN) staining has limited sensitivity and depends on operator interpretation. We evaluated modified ZN protocols adapted for fluorescence microscopy to improve bacillary detection while preserving diagnostic performance. We first standardized modified ZN staining conditions and identified protocols 3 and 4, both incorporating potassium permanganate, as the most suitable for fluorescence-based reading, whereas protocols without heat or with prolonged dye exposure showed suboptimal visualization or increased background noise. We then compared bacilloscopy readings across methods, including MINSA conventional ZN, modified ZN protocols, fluorescent ZN, auramine staining, and GeneXpert MTB/RIF. Inter-reader comparison between DIRESA-Norte and our laboratory showed moderate agreement (κ=0.5091), strong ordinal correlation (ρ=0.7863), and no systematic differences (Wilcoxon p=0.1591). Under optical microscopy, protocols 3 and 4 showed high agreement with the MINSA protocol (κ=0.8387 and 0.8715, respectively), strong correlation in bacillary load classification (ρ=0.8806 and 0.8934), and no significant systematic bias. Under fluorescence microscopy, MINSA-fluorescent, protocol 3 fluorescent, and protocol 4 fluorescent also showed strong correlation with conventional bacilloscopy, with evidence of improved bacillary detection, particularly in high-load samples. Against GeneXpert, fluorescent ZN showed sensitivities of 0.83 for MINSA, 0.75 for protocol 3, and 0.86 for protocol 4, with specificities of 1.00, 0.98, and 0.98, respectively. Against auramine, fluorescent ZN showed sensitivities of 0.81, 0.74, and 0.84, and specificities of 0.98, 0.98, and 0.96 for MINSA, protocol 3, and protocol 4, respectively. These findings support fluorescent ZN as a promising alternative for tuberculosis smear microscopy, with modified protocols offering good diagnostic performance and operational advantages for fluorescence-based detection.

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IS THE INFLAMMATORY MARKER NEUTROPHIL-LYMPHOCYTE RATIO A PROGNOSTIC TOOL TO PREDICT SEVERITY AND MORTALITY IN COMMUNITY ACQUIRED PNEUMONIA IN A REAL LIFE SETTING?

Jimena Pérez García[1], Beatriz Villegas-Lara[2], Jorge Escobedo De La Pena[3]
[1]Universidad Nacional Autonoma de Mexico, Mexico City, Mexico, [2]Instituto Mexicano del Seguro Social, Mexico City, Mexico, [3]IMSS, Mexico City, Mexico

Community acquired pneumonia is a well-recognized major infectious cause of morbidity and mortality and having access to quick and reliable prognostic indexes is still a challenge. We conducted a follow-up study among hospitalized subjects with community acquired pneumonia to assess real-life reliability of the neutrophil-lymphocyte index (NLI), while compared to the CURB65 score, to predict mortality during hospitalization. We hypothesize that a high NLI was associated with a high mortality risk. We enrolled 572 patients hospitalized in a second-care regional hospital, both in ward and in an intensive care unit, from January 2024 to March 2026 in Mexico City. The first complete blood count obtained on admission was used to estimate the neutrophil-lymphocyte ratio (NLR) by dividing the absolute number of neutrophils by the absolut ecount of lymphocytes. The CURB65 score was also estimated. Case fatality rate (CFR) during hospitalization was estimated according to tertiles of the NLI as well as CURB65 score. Chi square test was used to assess association. The participants median age was 71 years (IQR 56-83) and 51.6% were

females. The identified or suspected etiologic agent was bacterial (62.4%), viral (16.1%), unspecified (19.9%) or other (1.5%). The CFR was 21%, lower in the first NLI tertile (13.2%), intermediate in the second tertile (18.3%) and higher in the third tertile (31.4%) (Chi-square 20.4; p<0.001). NLI discrimination capacity was slightly better in females (CFR per tertile 12.3%, 18.9% and 33.7%) that in males (CFR per tertile 14.5%, 17.7% and 29.5%), and when a bacterial etiology was suspected or identified (CFR per tertile 12.0%, 16.5% and 32.1%). The NLI was slightly useful to predict invasive respiratory support (IRS). The rate of IRS was 8.9% in the first NLI tertile, 13.1% in the second and 17.8% in the third NLI tertile (Chi-square 6.5, p= 0.04). The CFR in CURB65 ranged from 10.5% (score 0-1), to 22.7% (score 2) and 49.5% (score ≥3) (Chi-square 64.6; p<0.001). NLI is a reliable prognostic score for intrahospital fatality, like CURB65 although easier and faster to be obtained on admission.

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FUNCTIONAL CHARACTERIZATION OF RV0191 AND RV1258C IN PYRAZINOIC ACID EFFLUX AND PYRAZINAMIDE SUSCEPTIBILITY IN *MYCOBACTERIUM TUBERCULOSIS* BY GENETIC COMPLEMENTATION

Frank Anthony Pizarro Rojas, Patricia Sheen Cortavarria, Mishell Landa Flores

Cayetano Heredia Peruvian University, Laboratory of Bioinformatics, Molecular Biology and Technological Developments, San Martin de Porres, Lima, Peru

Pyrazinamide (PZA) is a key antibiotic in the therapeutic regimen for tuberculosis; however, the emergence of resistance to this drug represents a major obstacle to disease control. Traditionally, this resistance has been linked to mutations in the *pncA* gene, which inactivate the enzyme pyrazinamidase and prevent the conversion of PZA into its active form, pyrazinoic acid (POA). Nevertheless, PZA-resistant isolates lacking alterations in *pncA* have also been described, suggesting the involvement of alternative resistance pathways. Growing evidence indicates that efflux systems may contribute to this phenomenon by actively exporting POA and reducing its intracellular accumulation, thereby decreasing treatment efficacy. In this context, the genes *Rv1258c* and *Rv0191* have been proposed as possible efflux pumps involved in PZA resistance. The present study evaluates the specific function of *Rv1258c* and *Rv0191* through genetic complementation in *Mycobacterium tuberculosis* H37Rv knockout strains using the integrative plasmid pMV306hsp. Genetic complementation makes it possible to confirm the causal relationship between gene loss and the observed phenotype by determining whether reintroduction of the gene restores wild-type characteristics. We will assess whether complementation of these genes restores the H37Rv phenotype in terms of POA efflux rate, PZA susceptibility, and gene expression levels.

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ECONOMIC IMPACT OF ACUTE RESPIRATORY ILLNESS AMONG AGRICULTURAL WORKERS IN RURAL GUATEMALA

Gabriella J. Ess[1], Neudy Carolina Rojop[2], Diva M. Calvimontes[3], Julio David del Cid-Villatoro[4], Wanda Mejia[5], Kathryn Giron[5], Kareen Arias[5], Ingrid L. Contreras[6], Ashley Fowlkes[7], Chelsea Iwamoto[7], Guillermo Bolanos[5], Edwin Asturias[8], Molly Lamb[9], Daniel Olson[10]

[1]University of Colorado School of Medicine, Aurora, CO, United States, [2]Quetzaltenango, QZ, Guatemala, [3]FUNSALUD, Retalhuleu, Guatemala, [4]Fundación para la Salud Integral de los Guatemaltecos, Coatepeque, QZ, Guatemala, [5]Center for Human Development, Fundación para la Salud Integral de los Guatemaltecos (FSIG), Retalhuleu, Guatemala, [6]Center for Health Studies, Universidad del Valle de Guatemala, Guatemala City, Guatemala, [7]Centers for Disease Control and Prevention, Atlanta, GA, United States, [8]University of Colorado, Aurora, CO, United States, [9]Colorado School of Public Health, Aurora, CO, United States, [10]Univ of Colorado, Aurora, CO, United States

Respiratory illnesses have a high economic burden, an outcome that is particularly important to study in low-resource settings. In a prospective cohort of banana plantation workers in southwest Guatemala, workers reporting acute respiratory illness (ARI) (defined as self-reported fever or measured temperature >38°C, cough, or shortness of breath with onset in the last 7 days) were tested for influenza, RSV, and SARS-CoV-2 by RT-PCR and completed a follow up survey 7 and 28 days after illness onset. Economic data, including direct costs of illness and indirect costs (absenteeism), were collected from those with ARI and from a subset of randomly selected healthy workers (15 per week). Mean cost differences between ill and healthy workers were compared using the Student's t-test. The risk ratio (RR) of taking out a loan by day 28 of illness among workers with ARI compared with healthy workers was evaluated using a multivariable generalized linear regression model controlling for sex, worker monthly income, and food insecurity reported at enrollment. From June 2020 to October 2024, 2,809 workers were enrolled and followed; 425 met criteria for ARI, of which 31 (7.3%) and 96 (22.6%) tested positive for influenza and SARS-CoV-2, respectively. ARI resulted in 725 cumulative lost workdays, with a mean self-reported cost of $41.86 per household. Compared with the mean household monthly income of $510.01, the mean household cost of ARI due to SARS-CoV-2 was $95.45 and $38.19 for ARI due to influenza. Workers with ARI reported spending less money in the previous 7 days on meat compared to healthy workers ($24.18 vs $28.87, p≤0.01) and food insecurity was associated with taking out a loan at both day 7 (RR=2.86, p≤0.01) and day 28 (RR=1.99, p≤0.01) of illness. In this population of economically vulnerable agricultural workers, we identified a high economic burden of ARI, demonstrating the potential benefit of preventative interventions, including vaccination.

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STAKEHOLDER PERSPECTIVES ON THE INTRODUCTION OF A NOVEL TB VACCINE FOR ADOLESCENTS AND ADULTS IN PAKISTAN

Zoyia Hassan[1], Nazia Ahsan[2], doyoon kim[3], Rebecca Clark[4], Richard G White[4], Abdul Momin Kazi[5], Kristin Nelson[6]

[1]Aga Khan University Hospital, Karachi, Pakistan, [2]Aga Khan University, Karachi, Pakistan, [3]Emory university, Atlanta, Pakistan, [4]London School of Hygiene and Tropical Medicine, London, United Kingdom, [5]Aga Khan University, Karachi, SD, Pakistan, [6]Emory university, Atlanta, GA, United States

Pakistan is among the top five tuberculosis (TB) burden countries globally, and current prevention strategies remain insufficient. Novel TB vaccines for adolescents and adults are in the pipeline, but evidence on their implementation is limited. This study explored stakeholder perspectives on acceptability, feasibility, prioritization, delivery, and expected uptake in Pakistan using in-depth interviews analysed through thematic analysis with a hybrid deductive-inductive approach. All participants (n=10) expressed a clear need for a TB vaccine given the high disease burden, while emphasizing that support depends on evidence of safety, efficacy, and operational feasibility. Major acceptability challenges included low perceived risk of TB infection and persistent stigma, with participants noting that the absence of symptoms reduces urgency to vaccinate and that stigma discourages engagement with prevention. Participants emphasized that community engagement, provider-led communication, and endorsement by political and religious leaders would be critical to increase uptake. Health system readiness emerged as a key barrier, particularly related to workforce capacity, infrastructure gaps, and sustainable financing, with concerns about staffing shortages, limited facility readiness for adult vaccination, and uncertainty around long-term funding. A phased rollout was recommended, beginning with household contacts of TB patients as the initial priority group, while people living with HIV and adolescents were identified as priority groups for later phases. Coverage was expected to be modest initially, with most participants estimating uptake to be below 50%, increasing gradually with trust-building and program visibility. These findings suggest that TB vaccine introduction should prioritize household contacts as an initial strategy, alongside early investment in communication, trust-building, and delivery systems to support gradual scale-up in high-burden settings.

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UNRAVELING OVEREXPRESSION EFFECTS OF FIVE CANDIDATE EFFLUX PUMPS ON PYRAZINOIC ACID EFFLUX KINETICS AND PYRAZINAMIDE SUSCEPTIBILITY IN *MYCOBACTERIUM TUBERCULOSIS H37RV*

Kensy Brigit Atachahua Bravo, Maria Jose Jaico Román, Carlos Alonso Flores Bancayan, Patricia Sheen Cortavarria
Universidad Peruana Cayetano Heredia, Lima, Peru

Tuberculosis caused by Mycobacterium tuberculosis infection remains a major global health burden, particularly in low- and middle-income countries. In Peru, high rates of multidrug-resistant TB (MDR-TB) increasingly compromise treatment efficacy, especially due to resistance to pyrazinamide (PZA). This prodrug plays a critical role in shortening TB therapy and requires intracellular conversion to its active form, pyrazinoic acid (POA), by the pyrazinamidase encoded by pncA. While resistance to PZA is classically associated with mutations in pncA, resistant clinical isolates lacking such mutations highlight alternative mechanisms. Efflux-mediated extrusion of POA has emerged as a plausible mechanism reducing intracellular drug accumulation and bactericidal activity. In M. tuberculosis, several candidate efflux pumps, including Rv0191, Rv1258c, Rv3008, Rv1667c, and Rv3756c, have been implicated; however, their functional contribution remains poorly resolved. This study aims to assess the impact of overexpression of these five efflux pump genes on POA efflux dynamics, extracellular POA concentration, and PZA susceptibility in the H37Rv strain under acidic in vitro conditions (pH 6.2), mimicking the host intracellular environment. To achieve this, each gene will be overexpressed using an inducible episomal system (pNIT) activated by isovaleronitrile. POA efflux rates will be quantified using a modified Wayne assay, while drug susceptibility will be evaluated via the Tetrazolium Microplate Assay (TEMA). We expect that overexpression will increase POA efflux velocity, enhance extracellular POA accumulation, and correlate with reduced susceptibility to PZA. Furthermore, differential contributions among pumps may suggest functional heterogeneity. Overall, this study seeks to provide mechanistic insight into non-canonical pathways of PZA resistance, supporting a model in which efflux activity modulates intracellular POA homeostasis.

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FROM LABORATORY TO URBAN AND PERI-URBAN DEPLOYMENT: CHALLENGES IN SCALING SPATIAL REPELLENT TECHNOLOGIES IN BRAZIL

Tales Ribeiro[1], Álvaro Eiras[1], Fredros Oketch Okumu[2]
[1]Federal University of Minas Gerais, Belo Horizonte, Brazil, [2]Ifakara Health Institute, Ifakara, United Republic of Tanzania

Long lasting spatial repellents offer an attractive complementary strategy to reduce mosquito – human contact, yet few low-cost solutions have been validated under real-world conditions. This study evaluated repellent-impregnated footwear designed to provide continuous spatial protection against mosquito vectors of human disease in urban areas in Brazil. The project was conducted through a partnership between Federal University of Minas Gerais (Brazil) and the Ifakara Health Institute (Tanzania) between 2017 and 2019. Laboratory assays under controlled airflow showed ~70% immediate knockdown of *Aedes aegypti* exposed to treated textile strips. Semi-field evaluations demonstrated distance-dependent effects, with mosquito mortality ~40% at 1.5 m from the repellent source, whereas blood-feeding inhibition persisted for up to 12 hours after exposure, while fecundity and fertility were not affected. Olfactometer assays confirmed behavioral disruption of host-seeking females. Prototype repellent sandals (400 pairs) were manufactured, distributed and evaluated for 9 weeks in urban area in Belo Horizonte city (30,17 ha., 60 houses) for *Ae. aegypti* and peri-urban area in Porto Velho (10,35 ha., 60 houses) for *Anopheles darlingi*, the main malaria vector in Brazil. The results showed 69% protection against *Ae. aegypti* and 75% against *An. darlingi*. The user acceptability was high, 84% of participants in urban and 62% in peri-urban areas, expressed willingness to purchase the footwear. Interestingly, 80.7% were still using the sandals six months after the trial. These results demonstrate the feasibility, effectiveness, and social acceptability of low-cost repellent footwear as a scalable tool for urban and peri-urban vector control. Further technology improvements are on-going.

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BITEBARRIER AS A VOLATILE PYRETHROID SPATIAL EMANATOR FOR PROTECTION AGAINST VECTOR BORNE DISEASES IN OUTDOOR AND HIGH RISK POPULATIONS

Neil F. Lobo
Univ of Notre Dame, South Bend, IN, United States

Vector-borne diseases account for ~17% of the global infectious disease burden, with malaria, dengue, and visceral leishmaniasis causing substantial morbidity and mortality each year. However, primary tools, LLINs and IRS, are designed for indoor use and leave gaps in protection both indoors when not under nets and outdoors. These gaps are most pronounced among migrant workers, forest-dwelling communities, and military personnel, where exposure occurs in temporary shelters and open-air environments. We present results from a multi-country evaluation of BiteBarrier (BB; PIC Corporation), a transfluthrin-based volatile pyrethroid spatial emanator, across diverse ecological and transmission settings. In semi-field studies at Ifakara Health Institute, Tanzania, BB maintained high indoor protective efficacy (>80-95%) against *Anopheles gambiae*, *An. gambiae* (kdr), and *An. funestus* over eight weeks, with meaningful outdoor protection. In Thailand, BB significantly reduced landing, blood feeding, knockdown, and mortality in *Anopheles minimus* across two independent systems. Field studies support these findings. In Cambodia, BB reduced *Anopheles* landing by 95% in forest settings and achieved near-universal uptake across 27 villages, exceeding use of topical repellents and treated clothing. In Indonesia, BB reduced recapture rates by 75% in tribal shelters and achieved >3-fold reductions in tent trials. In Zambia, deployment in outdoor kitchen shelters resulted in 65-75% reductions in *Anopheles* landing.Across sub-Saharan Africa and Southeast Asia, BB demonstrated consistent efficacy in indoor, peridomestic, and outdoor environments. These findings support spatial emanators as a practical tool to address residual transmission in underserved, outdoor-exposed populations.

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UNDERSTANDING THE IMPACT OF TRANSFLUTHRIN PASSIVE EMANATORS ON DENGUE VIRUS TRANSMISSION, PUERTO RICO, 2024-2025

Forrest K. Jones[1], Rafael Angel Saavedra Hernandez[2], Samuel Lacen Boria[2], Parker K. Acevedo[1], Jomil M. Torres[3], Miladys Perez Velez[3], Laura Adams[1], Maile B. Thayer[1], Carol Y. Liu[1], Michel Alejandro Alejandro[4], Alberto J. Millan Melendez[4], Charon Gwynn[2], Eleanor N. Field[5], Saul Lozano[6], Ryan R. Hemme[1], Roberto Barrera[1], Julieanne Miranda-Bermúdez[2], Gabriela Paz-Bailey[1], Grayson Brown[2], Stephen H. Waterman[1]
[1]CDC Dengue Branch, San Juan, Puerto Rico, [2]Puerto Rico Vector Control Unit, San Juan, Puerto Rico, [3]Puerto Rico Department of Health, San Juan, Puerto Rico, [4]Puerto Rico Science, Technology, and Research Trust, San Juan, Puerto Rico, [5]Virginia Department of Health, Richmond, VA, United States, [6]CDC Arboviral Diseases Branch, Fort Collins, CO, United States

Passive emanators (PEs), also known as spatial repellents, are a promising vector control tool designed to reduce human-mosquito contact through the slow release of low concentrations of an insecticide (e.g., transfluthrin). During the 2024-2025 dengue outbreak in Puerto Rico, PE distribution was targeted to a 14.2 km^2 area with historically high dengue case burden. We compared dengue incidence between areas with varying levels of PE coverage. PEs were distributed through at-home installation, at-home delivery, and distribution events. While all residents in the high-incidence area could pick up PEs at distribution events, at-home installation and at-home delivery occurred in a subset of neighborhoods. Dengue cases were detected through routine case surveillance by the Puerto Rico Department of Health; only cases geocoded to the street-level were included. During October 2024-May 2025, we distributed 54,752 PEs to 5,450 households (10 PEs per household) across 99 neighborhoods. At-home installation

occurred at 664 (12%) households, at-home delivery occurred at 3790 (70%) households, 995 (18%) households picked-up PEs at distribution events, and distribution method was not recorded for 1 household. In 19 neighborhoods where at-home installation and at-home deliveries took place, we distributed PEs to >50% of households. We also identified 80 nearby low-coverage neighborhoods (median percent of homes receiving PEs: 2.9%, IQR: 0.0-3.3%). During June-November 2025, we identified 21 dengue cases in neighborhoods with high coverage and 41 cases in neighborhoods with low coverage. Dengue incidence was similar between high coverage neighborhoods (1.12 per 1000 population) and low coverage neighborhoods (0.95 per 1000 population). Before the dengue outbreak ended, PEs were deployed at a high level of coverage in some neighborhoods, primarily through at-home delivery. We found little difference in dengue risk between neighborhoods with and without high levels of PE coverage. Further research is needed to evaluate both the magnitude and duration of effectiveness of PEs in preventing dengue virus infections.

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THE EVALUATION OF THE SUMIONE SPATIAL EMANATORS IN EXPERIMENTAL HUTS IN SIAYA, WESTERN KENYA

Seline Omondi[1], Silas Okoth Agumba[1], Mathew Kipsum[1], Vincent Moshi[1], Bernard O. Abong'o[1], Brian Otieno Polo[1], Jackline Kosgei[1], John Gimnig[2], Ashley Hudson[3], Nicole L. Achee[3], Eric Ochomo[1]

[1]Kenya Medical Research Institute, Kisumu, Kenya, [2]CDC, Atlanta, GA, United States, [3]University of Notre Dame, Notre Dame, IN, United States

Spatial Emanators (SEs) are a promising vector control approach that could supplement existing malaria control tools. This study evaluated the efficacy of SumiOne™ SE, containing 10% metofluthrin, in experimental huts in Siaya, western Kenya. We evaluated the reduction in human landing rates, density, knockdown, and mortality rates of *Anopheles* mosquitoes. SumiOne™ SE significantly reduced indoor *Anopheles* landing rates by 59% compared to control huts (IRR = 0.41, 95% CI 0.233-0.711, P = 0.0063). For outdoor effects, there was a 44% reduction (IRR = 0.56, 95% CI 0.31-1.00, P = 0.0702) in *An. funestus*. When combining both indoor and outdoor data, SumiOne™ SE was associated with a significant 53% reduction in *Anopheles* landing rates (IRR = 0.47, 95% CI 0.268-0.824, P = 0.0195). The rate of *Anopheles* mosquitoes exiting the control huts was 7% (N=20) against 38% (N=99) for the intervention huts, indicating a significantly higher exiting rate from the intervention huts ($p < 0.0001$). Blood-feeding rates among collected *Anopheles* mosquitoes were significantly higher in control huts (77%) compared to 41% in the intervention huts ($p < 0.0001$). These findings support SumiOne™ SEs potential as a complementary vector control tool, alongside existing core interventions such as Insecticide Treated Nets and Indoor Residual Spraying, particularly in contexts where additional protection is needed.

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TECH ENABLED LARVAL SOURCE MANAGEMENT (LSM) FOR THE CONTROL OF *ANOPHELES STEPHENSI* IN EASTERN ETHIOPIA

Dorian Michael Jackson[1], Gonzalo Vazquez-Prokopec[2], Solomon Yared[3], Araya Gebresilassie[4], Esayas Aklilu[5], Huan Ning[1], Xiao Huang[1], Shoibolina Kaushik[1]

[1]Emory University, Department of Environmental Science, Decatur, GA, United States, [2]Emory University, Atlanta, GA, United States, [3]Jigjiga, Ethiopia, [4]Addis Ababa, Ethiopia, [5]Addis Ababa, United States

Vector control in sub-Saharan Africa has largely relied on insecticide-treated nets and indoor residual spraying to reduce rural malaria. However, the emergence and rapid spread of the urban vector *Anopheles stephensi* since 2016 threatens this progress. *An. stephensi* favors artificial, man-made breeding sites and bites earlier in the evening, reducing the effectiveness of current interventions and enabling urban malaria transmission. Additionally, during a recent malaria outbreak in Dire Dawa, Ethiopia, cases coincided with sites positive for *An. stephensi* larvae, implicating the vector in urban transmission. We provide results from a two-year entomological survey across multiple Ethiopian cities to assess the distribution of *An. stephensi* and report on the use of such findings to guide the deployment of residual larvicides in superproductive habitats using satellite imagery and AI algorithms. During the dry season, approximately 77% of *An. stephensi* larvae were concentrated in roughly 23% of surveyed habitats. The vast majority (>95%) of such superproductive habitats were cisterns used for construction purposes. A mathematical model predicted that focused larval source management (LSM) in such productive habitats may substantially reduce- or potentially eliminate- *An. stephensi* from Ethiopian cities, provided such habitats can be easily detected. Building on these findings, we will conduct an integrated, spatially targeted, LSM program across three Ethiopian urban centers (Jigjiga in the Somali region, as well as Semera and Logiya in the Afar region). We report our goal of combining high-resolution satellite imagery with geo-AI algorithms to identify likely *An. stephensi* breeding sites and deploy long-lasting larvicides (Spinosad and pyriproxyfen) for targeted habitats. Here, we present results of a pilot implementation of this tech-enabled LSM method on *An. stephensi* larval productivity and adult abundance during a 6-month period.

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LARVAL ECOLOGY OF THE INVASIVE *ANOPHELES STEPHENSI* IN MARSABIT COUNTY, NORTHERN KENYA; IMPLICATIONS FOR LARVAL SOURCE MANAGEMENT

Don Omondi[1], Patriciah Mbithe Wambua[1], Cyprian Adala[2], Yohana Mwalugelo[3], Esther Osir[1], Racheal Nyambura[1], Kelvin Kioko[1], Jared Oremo[1], Erick Odhiambo Muok[4], Shirley Akinyi Onyango[5], Patience Kiyuka[6], Stephen Odhiambo Munga[7], Jean-Philippe Parvy[8], Emilie Pondeville[8], **Shehu Shagari Awandu**[1]

[1]Jaramogi Oginga Odinga University of Science and Technology, Bondo, Kenya, [2]Kenya Medical Research Institute- Center for Global Health Research, kisumu, Kenya, [3]Ifakara Health Institute, Dar Es Salaam, United Republic of Tanzania, [4]Kenya Medical Research Inst, Kisumu, Kenya, [5]International center of excellence for malaria reearch, Homa Bay, Kenya, [6]Kenya Medical Research Institute, Kenya, [7]Kenya Medical Research Institute, Kisumu, Kenya, [8]MRC - University of Glasgow Centre for Virus Research, Glasgow, United Kingdom

The recent African invasion of *Anopheles stephensi* that thrives in urban environments poses a significant challenge. Models predict that it will place an additional 126 million people at risk and introduce new transmission challenges if its spread is left unchecked. While the species is linked to malaria outbreaks, little is known about its ecology in most of Africa that limits its containment. In line with the WHO vector alert for increased surveillance and to inform larval control strategies, we conducted larval surveillance for *An. stephensi* in urban settings of Laisamis and rural regions of Moyale in Marsabit county, Kenya from March 2025 -January 2026. We sampled 257 different habitats, more during the rainy season than during the dry season with the majority of habitats man-made. The most productive natural habitats were river flood plains while hoof-prints were the most productive artifical habitats. Anopheles mosquites were most dominant in artifical habitats. Molecular analyses and sequencing revealed the presence of *An. stephensi* from over half of the larval samples from Laisamis (56.6%, 23/41). Using a Random Forest model, larval presence was primarily driven by physicochemical factors, with temperature emerging as the strongest predictor, followed by pH and turbidity. Nonlinear responses showed that larval occurrence increased from ~0.20 at lower temperatures to peak probabilities of 0.70-0.80 within an optimal range of ~28-32°C, before declining at higher temperatures. Similarly, occurrence was highest under neutral to slightly alkaline conditions (pH ~7.0-8.5), decreasing by over 30% outside this range. Turbidity had a negative effect, reducing predicted presence by ~0.15-0.25 across the gradient. Interaction effects indicated that optimal combinations of moderate temperature and neutral pH more than doubled predicted occurrence compared to suboptimal conditions. The persistence of stable *Anopheles stephensi* larval populations in northeastern Kenya highlights the potential effectiveness of targeted larval source management in fixed and identifiable habitats to limit the spread of this invasive vector.

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A NOVEL SUIT OF PROTOTYPE MICROBIAL BIOPESTICIDES FOR VECTOR CONTROL

George Dimopoulos

Johns Hopkins University Bloomberg School, Baltimore, MD, United States

Chemical insecticide resistance in mosquitoes remains a formidable barrier to global disease control efforts, necessitating the discovery of sustainable alternatives with novel modes of action. Microbes naturally synthesize a diverse array of bioactive molecules in response to ecological pressures, many of which harbor significant potential for insect pest management. In this study, extensive environmental sampling across diverse Mediterranean, Caribbean, and Central American habitats resulted in the assembly of microbial libraries comprising approximately 2,150 bacterial isolates. High-throughput screening of roughly 1,000 of these isolates, utilizing both heat-inactivated and sonication-inactivated cultures, facilitated the identification of specific strains producing potent mosquito larvicidal compounds. Through a comprehensive selection process evaluating potency, activity spectrum, and environmental stability, 15 highly promising candidates were identified from a variety of microbial species. Detailed biochemical and genomic investigations revealed that these candidates produce novel insecticidal secondary metabolites and proteins. Notably, several of these molecules demonstrated dual activity, effectively targeting both larval and adult mosquito stages, as well as broader insect pests. To translate these findings into field-ready solutions, innovative delivery systems were developed, including specialized pellet formulations for larval control and attractive toxic sugar baits (ATSB) for adult management. Crucially, leading candidates confirmed high efficacy against established insecticide-resistant mosquito populations and further demonstrated a negative impact on vector competence. These results highlight the immense value of microbial-derived metabolites as innovative tools for integrated and sustainable disease vector management strategies globally.

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COMPARATIVE EFFECTIVENESS OF FIVE WHO RECOMMENDED INSECTICIDES FOR VECTOR-BORNE DISEASE PREVENTION WHEN USED FOR INDOOR RESIDUAL SPRAYING OF TEMPORARY REFUGEE SHELTERS CONSTRUCTED OF UNHCR PLASTIC SHEETING

Richard James Allan[1], Svetlana Ryazanskaya Randle[2], Louise Ford[3], Kyle Walker[3], Stephanie Herodotou[2], Mohammad Kamal[4], Sajjad Ahmad[4], Adriana Adolfi[3], Jason H. Richardson[2], Michael Macdonald[5]

[1]The MENTOR Initiative, Haywards Heath, United Kingdom, [2]IVCC, Liverpool, United Kingdom, [3]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [4]The MENTOR Initiative, Maiduguri, Nigeria, [5]Innovative Vector Control Consortium (IVCC), Catonsville, MD, United States

The number of people forced displaced by conflict or extreme weather, living in temporary shelter of various forms, for up to 10 years, will likely exceed 136 million in 2026. Transmission of vector borne diseases ramps up quickly under these conditions. Protecting vulnerable people is critical to saving lives in humanitarian crises. Indoor residual spraying (IRS) is recommended by WHO for malaria control in permanent and temporary shelters. However, insecticide resistance in mosquito populations against pyrethroids has forced the adoption of new insecticide active ingredients (AI). A MENTOR/IVCC/LSTM partnership studied the effectiveness of WHO recommended A.I. on shelters made of plastic sheeting under refugee camp conditions. FICAM® VC WP 80 (bendiocarb); ACTELLIC® 50 EC (pirimiphos-methyl); Fludora® Fusion WP (clothianidin + deltamethrin); SumiShield® 50 WG (clothianidin); and VECTRONTM T500 (broflanilide) were tested for 5 months. Test and control shelters, were sampled (30 x 30cm pieces from shelter bottom, mid and upper) monthly. LSTM, conducted bio-assays (Kisumu strain), HPLC analysis, and sedimentation tests. Mosquito mortality of around 100% was observed using ACTELLIC®, Fludora® Fusion and VECTRONTM T500 across the study period. FICAM® resulted in 100% mortality for three months. It's efficacy dropped sharply thereafter. SumiShield® fell below the WHO effectiveness cut-off point of 80% mortality after just one month. HPLC demonstrating relatively high levels of AI in whole samples sprayed with Sumishield. Sedimentation rates for AI (except VECTRONTM T500) measured over 60 minutes using TURBISCAN AGS revealed that SumiShield® resulted in the thickest sedimented layer indicating that it's AI particles are heavier, and sediment more quickly out of water, compared to other AI. This study provides strong evidence that ACTELLIC®, Fludora® Fusion, and VECTRONTM T500 are highly effective on temporary shelters made of plastic sheeting. Use of FICAM® (the oldest of the five products), would require a second IRS round mid-way through typical wet season durations. Sumisheild® proved ineffective on plastic sheeting.

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PORTABLE EXPERIMENTAL HUT TENTS (PEHTS): A NOVEL FRAMEWORK FOR IMPROVING MALARIA VECTOR CONTROL DECISION-MAKING

Richard M. Oxborough[1], Trishan Wickramasinghe[1], Nikita Gopakumar[1], Abdul Rahim Mohammed Sabtiu[2], Whitney Qualls[3], Joseph Diclaro[3], Jaslyn Stamey[3], Rui-de Xue[3], Yafet Tiruha[1], Thomas S. Churcher[4], Seungman Park[1], Yaw A. Afrane[2], Louisa Alexandra Messenger[1]

[1]University of Nevada, Las Vegas, Las Vegas, NV, United States, [2]University of Ghana, Accra, Ghana, [3]Anastasia Mosquito Control District, St. Augustine, FL, United States, [4]Imperial College London, DIDE, London, United Kingdom

Insecticide-treated nets (ITNs) remain the primary malaria vector control tool across sub-Saharan Africa, with over 2.2 billion pyrethroid ITNs distributed since 2004. Widespread pyrethroid resistance has contributed to stalled progress, but new ITNs incorporating chlorfenapyr or piperonyl butoxide provide improved efficacy. With growing intervention options but limited resources, malaria programs face complex decisions influenced by local transmission intensity, vector species composition, and dynamic insecticide resistance patterns. Decisions often rely on sub-national bioassay data, which does not predict ITN field performance, particularly for non-neurotoxic insecticides like chlorfenapyr. Experimental hut trials are the gold standard for assessing ITN efficacy, but fixed huts are limited, often located near rice fields for consistent mosquito yields, and may not reflect high-burden village settings. We present portable experimental hut tents (PEHTs), modeled on West African-style huts, consisting of large cabin-style tents equipped with prototyped 3D-printed mosquito entry points. PEHTs can be deployed in areas of greatest burden such as Nigeria and the Democratic Republic of Congo, which accounted for 104 million (>37%) malaria cases in Africa in 2023, to allow sub-national tailoring of vector control interventions. They can be utilized to evaluate community-used ITNs of varying ages against local vector populations. Entomological data from PEHTs characterizing mosquito mortality, blood-feeding and repellency will feed into a mechanistic model of *P. falciparum* transmission dynamics. Beyond ITN evaluation, PEHTs provide a flexible platform for testing emerging vector control technologies such as spatial repellents and can generate evidence during humanitarian emergencies. Release-recapture experiments are ongoing to characterize mosquito behavior and validate PEHT performance. This novel framework offers a field-deployable, innovative approach for evaluating malaria vector control interventions across heterogeneous, high-transmission settings.

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UNIVERSAL IMMUNOTHERAPY AGAINST HANTAVIRUS: HUMAN MONOCLONAL ANTIBODIES GENERATED VIA THE HIS-DRAGA MOUSE TO SAFEGUARD GLOBAL PUBLIC HEALTH AGAINST EMERGING ZOONOTIC THREATS

Ahmad Faisal Karim[1], Sounak Ghosh Roy[1], Sofia Ana Casares[2]
[1]Henry M Jackson Foundation for the Advancement of Military Medicine, Silver Spring, MD, United States, [2]Naval Medical Research Command, Silver Spring, MD, United States

Hantaviruses represent a severe global public health threat, responsible for Hemorrhagic Fever with Renal Syndrome (HFRS) and the highly lethal Hantavirus Pulmonary Syndrome (HPS). Endemic to diverse tropical, subtropical, and temperate regions, these zoonotic pathogens currently lack FDA-approved medical countermeasures, leaving vulnerable populations at risk. Globally, an estimated 150,000 to 200,000 cases occur annually. “Old World” hantavirus strains primarily drive widespread HFRS outbreaks across Europe and Asia, while “New World” strains cause severe HPS throughout the Americas. Consequently, there is an urgent clinical need within tropical medicine for a pan-hantavirus immunotherapy capable of conferring broad-spectrum protection, a challenge that fully human monoclonal antibodies (hu-mAbs) are uniquely positioned to address. To overcome the limitations of conventional animal models, we utilized the Human Immune System (HIS)-reconstituted DRAGA mouse. Reconstituted with human cord blood stem cells, HIS-DRAGA mice mirror complex human immune responses, enabling the rigorous preclinical evaluation of medical countermeasures and providing an agile platform for rapidly generating therapeutic hu-mAbs. Utilizing this innovative *in vivo* surrogate system, we generated a diverse panel of hu-mAbs. Crucially, a significant number of these fully human antibodies exhibit broad cross-strain reactivity against the diverse hantavirus strains causing both HPS and HFRS, underscoring their medical significance for global deployment in heavily impacted endemic regions. By accurately modeling human viral pathogenesis and bridging the translational gap between animal studies and clinical trials, the HIS-DRAGA platform significantly accelerates the pipeline for broad-spectrum therapeutics. Moving forward, we propose utilizing combinatorial cocktails of these pan-hantavirus hu-mAbs to synergistically neutralize live hantavirus plaque formation. Beyond immediate outbreak response, this scalable technology paves the way for rapid countermeasure development against other emerging tropical viral threats.

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ABROGATING CXCR3 SIGNALING DECREASES ZIKA AND WEST NILE VIRUS REPLICATION

Jennifer Lynn Spencer Clinton[1], Afzila Bhojani[2], Daniel Rehm[1], JeAnna Redd[1], Alexander Kneubehl[1], Shannon Ronca[1]
[1]Baylor College of Medicine, Houston, TX, United States, [2]University of Houston, Houston, TX, United States

Flaviviruses such as Zika (ZIKV) and West Nile (WNV) cause >400 million infections annually and result in significant morbidities, including birth defects and adverse neurological outcomes. With climate change expanding geographic vector range, therapeutics are critically needed to prevent flavivirus infection and resultant disease. Flavivirus replication is regulated by host immune responses, including interferon-stimulated genes (ISG) and their receptors. The ISG IP-10 and its receptor, CXCR3, are upregulated during ZIKV and WNV infections and have been associated with antiviral activity. However, the role of IP-10 and CXCR3 during flavivirus infections is unknown. We previously demonstrated that blocking CXCR3 abrogated ZIKV replication in human prostate cells without altering cellular proliferation or survival. Therefore, we aimed to define CXCR3 antiviral effects against ZIKV and WNV across other sites of infection *in vitro* and *in vivo*. We confirmed CXCR3 expression on human monocytes by immunofluorescence. Next, we treated monocytes with CXCR3 antagonist prior to infection with ZIKV or WNV and assessed changes in ZIKV and WNV envelope expression by qRT-PCR. There were significant reductions in ZIKV and WNV envelope expression in antagonist-treated cells compared to untreated controls. *In vivo* studies have assessed CXCR3 antagonism in preventing WNV neurological disease, and preliminary data indicate blocking CXCR3 may delay disease onset and neuropathology. This work highlights an undescribed host factor that can restrict both ZIKV and WNV replication. Our findings indicate that CXCR3 antiviral activity applies to multiple flaviviruses and occurs in different cell types, indicating a potential conserved mechanism over multiple tropic sites. Future work will 1) elucidate the antiviral mechanism of CXCR3, including the potential to ameliorate cellular stress and 2) evaluate the bioavailability of CXCR3 antagonists *in vivo* to inform therapeutic potential. Better understanding the mechanism of CXCR3 flavivirus restriction could lead to the development of novel and cross-protective flavivirus therapeutics.

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ACCELERATING COUNTERMEASURES FOR EMERGING TROPICAL DISEASES: HUMAN MONOCLONAL ANTIBODIES AGAINST SEVERE FEVER THROMBOCYTOPENIA SYNDROME VIRUS (SFTSV) DERIVED FROM THE HIS-DRAGA MOUSE

Sounak Ghosh Roy[1], Ahmad Faisal Karim[1], Zhaodong Liang[2], Daniel F. Ewing[2], Jeffrey Robert Currier[3], Sofia Ana Casares[4]
[1]Henry M Jackson Foundation for the Advancement of Military Medicine, Silver Spring, MD, United States, [2]Naval Medical Research Command, Silver Spring, MD, United States, [3]WRAIR Walter Reed Army Inst of Research, Silver Spring, MD, United States, [4]Naval Medical Research Center, Silver Spring, MD, United States

Severe Fever Thrombocytopenia Syndrome Virus (SFTSV) is an emerging and highly lethal tick-borne bunyavirus that poses a critical global health threat, particularly in tropical and subtropical endemic regions, impacting both civilian populations and deployed allied forces. Developing effective medical countermeasures for such severe tropical and emerging infectious diseases requires robust and reliable preclinical models. The human-immune-system (HIS)-humanized DRAGA mouse serves as an advanced surrogate *in vivo* human model, facilitating the comprehensive investigation of infectious diseases that exclusively infect humans. This includes notable tropical threats such as *P. falciparum*, scrub typhus, dengue, Zika virus, Hantaan virus, alongside HIV, influenza, and COVID-19. Building upon previous success in isolating fully human monoclonal antibodies (hu-mAbs) against respiratory viruses, we leveraged this mouse model as an innovative preclinical platform for generating targeted hu-mAbs against SFTSV. The HIS-DRAGA platform was harnessed to uniquely isolate these human antibodies following immunization with SFTSV DNA and a subsequent targeted booster dose utilizing SFTSV recombinant protein. Comprehensive molecular characterization was performed to sequence the heavy and light chain variable regions, while structural integrity was confirmed via SDS-PAGE and Western blotting. We successfully generated a diverse panel of eleven IgG hu-mAbs reactive against SFTSV molecules. Individual and combinatorial cocktails of these therapeutic hu-mAbs were rigorously assessed for their ability to actively neutralize live SFTSV plaque formation *in vitro*. Crucially, the combinatorial cocktails of the generated hu-mAbs worked synergistically to achieve maximal neutralization of the live virus. These compelling results demonstrate that the HIS-DRAGA model is a highly effective preclinical platform for producing therapeutic hu-mAbs against SFTSV and other emerging tropical infectious diseases, ultimately accelerating critical countermeasure development for global public health and deployed force protection.

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LAMP-LFA FOR ARBOVIRAL POINT-OF-CARE DIAGNOSTICS: DESIGN OF A MOLECULAR TOOL FOR THE DETECTION OF DENGUE AND YELLOW FEVER

Cheikh Talibouya Toure
Institut Pasteur de Dakar, Dakar, Senegal

Arboviruses pose a significant public health threat, with over 5 million dengue cases and rising yellow fever (YF) outbreaks reported globally in 2025. In resource-limited settings, centralised PCR diagnostics hinder rapid

responses in rural areas, necessitating the development of decentralised point-of-care (POC) tools. This study describes the development and validation of a molecular diagnostic platform utilising Loop-mediated Isothermal Amplification (LAMP) coupled with a lateral flow assay (LFA). For YF, primers were sourced from the literature and the assay was validated using 24 strains from the Institut Pasteur de Dakar biobank, demonstrating 100% sensitivity and a limit of detection (LoD) of 10^-2 PFU (0.58 IU/mL), equivalent to that of the gold-standard PCR. To address the high genetic diversity of the dengue virus (DENV), we developed LAVA 2.0, an update of the LAVA software tool, through AI-assisted "vibe coding." Unlike traditional tools, LAVA 2.0 supports Multiple Sequence Alignment (MSA), degenerate nucleotides, and the design of stem primers to enhance reaction speed and coverage. Evaluation of 2,664 DENV-2 sequences yielded primer sets with an individual coverage of 80%, increasing to 93% when combined, while stem primer sets achieved 92% coverage with no significant dimerisation. These findings establish a highly sensitive POC tool for YF and provide a robust computational framework via LAVA 2.0 to streamline the development of molecular diagnostics for genetically diverse emerging pathogens in decentralised settings.

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IMMUNOASSAY OPTIMIZATION OF HUMAN MILK-DIRECTED ELISA METHODS FOR DETECTION OF IMMUNOGLOBINS TO HEV

Madeleine Blunt[1], Beverly Plunkett[1], Brittany Kmush[2], Kawsar R. Talaat[3]

[1]Johns Hopkins, Baltimore, MD, United States, [2]Syracuse University, Syracuse, NY, United States, [3]Johns Hopkins Univ, Baltimore, MD, United States

Hepatitis E disease can be fatal to pregnant women and their neonates. Transmission of antibodies through human breast milk (HBM) has been shown to protect infants from other pathogens after maternal vaccination. To understand the full impact of maternal HEV immunization, HEV antibodies in HBM will need to be measured but there is no commercially available ELISA kit validated for HBM. We optimized five ELISAs for the detection of anti-HEV IgG, IgM, and IgA in HBM. Experiments were carried out utilizing the Wantai anti-HEV IgG and IgM sera-directed ELISA kits. A checkerboard dilution method was used to assess assay performance across different concentrations of positive control in both sample diluent and HBM test samples. ELISAs were conducted using the package insert procedures with spiked sample diluent as a reference value. We demonstrated a high concordance of results between our optimized HBM- directed ELISAs and the commercially available sera-directed ELISA kit, indicating that we are now able to detect immunoglobins targeting HEV in HBM. However, we observed decreased anti-HEV IgM sensitivity for HBM samples containing high total IgM when using the commercially available assay kit. Modifying the assay to be performed using the IgG kit pre-coated plate and an external IgM conjugate increased the sensitivity of the assay and decreased the risk of false negatives among mothers who may have recently been ill or received another vaccine. Additionally, we were able to develop methodology to assess for anti-HEV IgA in HBM. This was significant, as IgA is a crucial antibody for understanding mucosal immunology and the impact of HBM on prevention of disease in neonates and infants. The impact of maternal HEV immunization on the fetal immune response and whether HEV antibodies in HBM can serve as an immunological correlate of protection for HEV still needs to be determined. The development of these assays will allow us to advance our ability to understand and characterize the immunological response to HEV 239 by detecting anti-HEV IgG, IgA and IgM in HBM after vaccination.

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HIGH-THROUGHPUT MULTIPLEX ASSAY FOR DETECTING QUATERNARY EPITOPE ENVELOPE ANTIBODIES AGAINST DENGUE VIRUS

Marielys Collazo-Roman, Jedas V. Daag, Ruby P. Shah, Aravinda de Silva, Prem Lakshmanane

University of North Carolina, Chapel Hill, NC, United States

The four dengue serotypes (DENV 1-4) cause substantial pediatric morbidity worldwide and are a significant cause of hospitalization in endemic countries. Quaternary epitope-directed antibodies against the envelope (E) dimer correlated with protection in previous studies, like Mpingabo et al. 2024, but simplified assays are needed to profile these antibodies across all dengue serotypes to advance dengue vaccine immunogenicity studies. We developed a multiplex assay using recombinant DENV1-4 E monomers and E dimers on the Meso Scale Diagnostics (MSD) platform to simultaneously detect quaternary E dimer-directed antibodies, requiring ~2 µL of serum. We validated the assay and antigens using a 13-panel of human monoclonal antibodies (mAbs). Concentration curves established assay performance and optimal antigen working concentrations. We used a competition protocol with wild-type monomers to deplete monomer-specific antibodies, detecting only quaternary dimer-specific antibodies. Incubating mAbs with excess E monomer antigen retained the binding signal for dimer-specific antibodies while reducing signal for monomer-directed antibodies, confirming that this competition step effectively depletes monomer-focused antibodies, allowing profiling of the E dimer antibody signal. As proof-of-concept, we tested non-human primate (NHP) sera with known vaccination status (monovalent DENV2 E-dimer, tetravalent stabilized E-dimer, or tetravalent wild-type E-protein vaccine) in the MSD assay and compared with the mature virus neutralization assay. E-dimer-specific signals were detected in NHPs vaccinated with E-dimer vaccines but not in those receiving wild-type E-protein vaccines, demonstrating the assay's ability to discriminate quaternary epitope responses. We have established a high-throughput, sample-sparing assay to detect antibodies directed against quaternary epitopes. This assay is well-suited to profiling quaternary immune responses in pediatric cohorts and in vaccine trials where sample volumes are limited. Evaluating a panel of human sera with known primary dengue infections is currently underway.

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PROTECTIVE AND ENHANCING ANTIBODIES AGAINST DENGUE VIRUS AMONG DENGVAXIA RECIPIENTS IN THE PHILIPPINES

Yu-Ching Dai[1], Ava Kristy Dy Sy[2], Mario Jiz[2], Joan Bato[2], Mary Ann Quinoñes[2], Mary Ann Joy Reyes[2], Wei-Kung Wang[1]

[1]John A. Burns School of Medicine, University of Hawaii at Manoa, Honolulu, HI, United States, [2]Research Institute for Tropical Medicine, Muntinlupa City, Metro Manila, Philippines

The 4 serotypes of dengue virus (DENV) are the leading cause of mosquito-borne viral diseases in humans, with >14 million cases reported in 2024. Dengvaxia, a chimeric yellow fever dengue vaccine, was licensed in 2016, followed by a revised recommendation for baseline DENV-immune individuals in 2018 due to higher risk of severe disease among baseline DENV-naïve individuals. After primary DENV infection, cross-reactive antibodies with weakly neutralizing activity have been implicated in antibody-dependent enhancement (ADE) and severe disease during secondary DENV infection. While Dengvaxia has been reported to boost neutralizing antibodies in baseline DENV-immune individuals, baseline DENV-naïve recipients may develop lower titers of neutralizing antibodies and cross-reactive antibodies, causing ADE during breakthrough DENV infection. Here, we determined baseline DENV serostatus using a previously reported DENV NS1 IgG ELISA (Dai et al. 2023), and examined neutralizing and enhancing antibodies among Dengvaxia recipients including baseline-naïve (n=21) and immune (n=24) groups. We found neutralizing antibody titers against four DENV serotypes were lower in baseline DENV-naïve group (P<0.001, each serotype, Mann-Whitney test), suggesting a weaker

protection compared with baseline immune group. Flow-based ADE assay revealed that most baseline DENV-naïve recipients can enhance DENV2 infection to the peak at 1:40 serum dilution, compared to 1:640 dilution in baseline immune recipients. Furthermore, the enhancing activities of baseline DENV-naïve sera at 1:10 dilution, a concentration close to real-world situations, is ~10-fold higher than that of baseline DENV-immune sera (P<0.0001, Mann-Whitney test), suggesting that baseline DENV-naïve sera are more likely to enhance DENV during breakthrough infection. Together, our findings demonstrate immunological mechanisms contributing to the low efficacy and high risk of severe disease observed in previous clinical trials. Future studies are needed to improve our understanding of immune responses induced by Dengvaxia and facilitate the development of dengue vaccines.

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MULTI-ANTIGEN BASED PEPTIDE ELISAS FOR *PAN*-EBOLAVIRUS SURVEILLANCE AND DIFFERENTIAL DIAGNOSIS

Sunil K. Dubey[1], Andrea Marzi[2], Ian Crozier[3], Richard T. Davey Jr.[4], Irina Maljkovic Berry[5], Zirui Zhou[1], Riddhi Thakkar[1], Shreyas Joshi[1], Dean Follmann[6], Olivier Tshiani Mbaya[3], H. Clifford Lane[4], W. Ian Lipkin[1], Hensley Lisa E.[7], Nischay Mishra[1]

[1]Center for Infection and Immunity, Mailman School of Public Health, Columbia University, New York, NY, United States, [2]Laboratory of Virology, Division of Intramural Research, National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health, Hamilton, MT, United States, [3]Clinical Monitoring Research Program Directorate, Frederick National Laboratory for Cancer Research, Frederick, MD, United States, [4]Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health, Bethesda, MD, United States, [5]Integrated Research Facility at Fort Detrick (IRF-Frederick), National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health, Frederick, MD, United States, [6]Biostatistics Research Branch, Division of Clinical Research, National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health, Bethesda, MD, United States, [7]National Bio and Agro-Defense Facility (NBAF), United States Department of Agriculture (USDA), Manhattan, KS, United States

Ebolavirus (EBOV) remain a major global health threat due to recurrent outbreaks, high mortality (25-90%), zoonotic transmission, and viral persistence in survivors. Serodiagnosis is confounded by cross-reactive antibody responses and widespread use of recombinant vesicular stomatitis virus (rVSV)-based vaccines for EBOV and fail to distinguish between vaccine-induced and infection-derived immunity. Current serological assays have limited utility in outbreak response, surveillance, and zoonotic risk assessment. We performed agnostic, epitope-resolved serology using a high-density peptide microarray (~391,000 overlapping,12-mer peptides) spanning *Filoviridae* proteomes, rVSV-EBOV vaccine antigens, and controls. Plasma samples included rVSV-EBOV-Glycoprotein (GP) and rVSV-Marburg virus-GP-vaccinated non-human primates (NHPs) challenged with EBOV with Makona strain, human vaccinees, Ebola virus disease (EVD) survivors, and acute EVD cases. Immunoreactive epitopes were identified using predictive classification models and validated by low-density arrays and ELISAs. Vaccinated NHPs and humans showed EBOV $GP_{1,2}$ and rVSV vector-specific responses, whereas post-challenge NHPs exhibited reactivity against $GP_{1,2}$, nucleoprotein (NP), VP35, and VP40. $GP_{1,2}$ epitopes consistently mapped to key functional regions, including the glycan cap, mucin-like domain, heptad repeat 2, and membrane-proximal external region. In vaccinees, antibody titers were age-dependent and increased after booster immunization. A panel of eight epitopes ($GP_{1,2}$, NP, VP40, VP35, and rVSV matrix protein) enabled discrimination between vaccination and natural infection and detected responses in acute EVD. Two conserved $GP_{1,2}$ epitopes have utility in *pan*-ebolavirus serodiagnosis. We demonstrate that proteome-wide epitope mapping can differentiate infection and vaccine-induced responses. We translated identified key epitopes into affordable multi-antigen-based peptide ELISAs for EBOV diagnostics, decentralized testing, outbreak response, and One Health surveillance.

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ANTI-NS1 ANTIBODIES DERIVED FROM YELLOW FEVER 17D VACCINATION MEDIATE INHIBITION OF ENDOTHELIAL BARRIER PERMEABILITY

Samantha R. Osman[1], Sarah M. Sampouw[1], Zhengchun Lu[1], Abram E. Estrada[1], Haley Brown[2], Peter Sullivan[2], Brian Booty[3], Evelin Coto[4], Tara Beatty[4], Allison Naleway[4], William B. Messer[1]

[1]Oregon Health & Science University, Portland, OR, United States, [2]Center for Infectious Disease Studies, OHSU-PSU, Portland, OR, United States, [3]Oregon Clinical and Translational Research Institute, Portland, OR, United States, [4]Kaiser Permanente Center for Health Research, Portland, OR, United States

Orthoflavivirus yellow fever virus (YFV) is endemic in regions of Africa/South America, with over 400M people estimated at risk of infection. While vaccination with the live-attenuated vaccine (LAV) YFV vaccine 17D induces neutralizing antibodies (NAbs) in most vaccinees, other immune mechanisms of protection likely exist. Orthoflaviviruses produce the viral non-structural protein 1 (NS1) that induces endothelial permeability when secreted from infected cells, contributing to disease pathogenesis. While NS1 specific mAbs have been extensively characterized in DENV infection, NS1 specific immune sera are understudied, and the presence and function of 17D elicited NS1 antibodies remain to be characterized. We hypothesized that LAV 17D induces anti-NS1 antibodies that can protect against NS1 mediated endothelial permeability. To address confounding by other orthoflavivirus infections common in 17D vaccinated populations, we compared NS1 immune sera between matched (sex, age, and years post-vaccination(ypv)) groups of 17D vaccinees with (n=19) and without (n=21) prior infection. We found the anti-NS1 antibodies are detectable in 75% of vaccinees, with no significant difference in titers between the two groups (Mann-Whitney p=0.5327). We next optimized a 17D NS1 trans-endothelial electrical resistance (TEER) assay for serum IgG, finding significantly lower AUC for 17D NS1 pre-treated with vaccinee IgG (n=40) versus naïve (n=20, Kruskal-Wallis p=0.001). Serum anti-NS1 antibody titers negatively correlated with AUC in TEER (Spearman r=-0.5928, p<0.001). We then evaluated anti-NS1 antibody kinetics in 55 naive vaccinees, finding peaks at D28 or 6-months post vaccination, stabilizing by 1 ypv. Acute peak viral load correlated with anti-NS1 titers at 1ypv (Spearman r=0.5956, p=0.0274), but acute secreted NS1 was rarely if ever detected. 1ypv IgG had significantly lower AUC compared to day 0 IgG in TEER (Kruskal-Wallis p=0.0332). These data are the first demonstration of the immunogenicity of 17D NS1 in humans and the functional capacity of human 17D anti-NS1 antibodies, supporting a secondary level of protection beyond NAbs.

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A KEY APIAP2 TRANSCRIPTION FACTOR ORCHESTRATES HOSTCELL REMODELLING IN *PLASMODIUM FALCIPARUM* INFECTED RED BLOOD CELL

Amit K. Subudhi[1], Sara Mfarrej[1], Rahul P. Salunke[1], Reem Abu-Shamma[1], Di Liu[1], Maya Aaych[2], David P.J. Ferguson[3], Sabrina Absalon[4], **Arnab Pain**[1]

[1]King Abdullah University of Science and Technology (KAUST), Pathogen Genomics La, Jeddah, Saudi Arabia, [2]King Abdullah University of Science and Technology (KAUST), Imaging and Characterisation CoreLab, Jeddah, Saudi Arabia, [3]University of Oxford, Oxford, United Kingdom, [4]Zionsville, IN, United States

Bloodstage *Plasmodium falciparum* extensively remodels infected red blood cells (RBCs), altering their permeability, rigidity and cytoadherence through the export of numerous parasite effector proteins to the RBC cytosol and membrane, a process central to malaria virulence. These exported effectors are expressed in a narrow temporal window, suggesting coordinated regulation by specific transcription factors. Here, we functionally characterise PfAP2HCR, an essential Apicomplexan AP2 (ApiAP2) DNAbinding protein in *P. falciparum* that operates during the intraerythrocytic developmental cycle and is a direct downstream target of the global regulator PfAP2P. PfAP2HCR, which contains two ApiAP2 domains, shows peak expression around 40 hours postinvasion,

coinciding with the period of intense hostcell remodelling. Using an inducible truncation system that conditionally removes both DNAbinding domains, we demonstrate that PfAP2HCR is required for early ringstage development, as loss of function causes parasites to arrest before the latering stage. RNAseq analysis of PfAP2HCRdeficient parasites reveals marked downregulation of genes encoding components of the Plasmodium Translocon of Exported Proteins (PTEX) and multiple exported effectors, indicating a critical role in controlling the export machinery and remodelling pathways. Complementary ChIPseq experiments show PfAP2HCR binding at the promoters of most of these hostcell remodelling genes, supporting their direct transcriptional regulation by this factor. Consistent with these molecular findings, transmission electron microscopy of pfap2hcrtruncated parasites demonstrates the loss of Maurer's clefts, providing structural evidence for a severe remodelling defect. Together, our data identify PfAP2HCR as a central transcriptional regulator that couples stagespecific gene expression to the biogenesis of export structures and effector delivery, thereby coordinating the hostcell remodelling that underpins *P. falciparum* pathogenicity.

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SINGLE-CELL SEQUENCING OF *PLASMODIUM VIVAX* BLOOD STAGE PARASITES FROM NATURAL INFECTIONS

Erin Sauve[1], Pieter Monsieurs[1], Katlijn De Meulenaere[1], Anna Caroline Campos Aguiar[2], Thafne Plastina Astro[2], Amália dos Santos Ferreira[3], Dhelio Batista Pereira[4], Carolina Bioni Garcia Teles[3], Roberto Rudge Moraes Barros[2], Anna Rosanas-Urgell[1]

[1]Institute of Tropical Medicine Antwerp, Antwerp, Belgium, [2]Federal University of São Paulo, São Paulo, Brazil, [3]FIOCRUZ Rondônia, Porto Velho, Brazil, [4]CEPEM, Porto Velho, Brazil

Single-cell RNA sequencing (scRNA-seq) is a powerful, high-resolution approach to study *Plasmodium* parasite biology and the cellular heterogeneity underlying its complex life cycle. To date, scRNA-seq studies of *P. vivax* blood stage parasites have been limited to non-human primate models, primarily due to the challenges associated with applying single-cell technologies in endemic settings. In this study, we collected and analyzed *P. vivax* blood stage single-cell transcriptomes from 9 natural infections from Porto Velho, Rondônia, Brazil. Out of the 9 natural infections, 5 clinical samples were preserved directly from the patients while 4 samples were cultured ex vivo prior to preservation. Single-cell RNA preservation and sequencing were done using HIVE CLX technology. For the analysis, conservative quality control filtering was applied with minimum thresholds of 500 genes/1000 transcripts per cell. After doublet removal, between 3,000-15,000 high-quality parasite transcriptomes per sample remained. We identified all asexual parasite blood stages; however, clinical and ex vivo-cultured parasites displayed enrichment in different life stages, with clinical samples being more enriched for early asexual life stages and the sexual stages, gametocytes. While gametocytes were found in both clinical and ex-vivo samples, some sexual stages were only observed ex vivo, thereby indicating likely stage-specific localization outside of peripheral blood. Additional insights into clinical and ex-vivo samples will be presented including stage-specific gene expression patterns and biomarker profiles. In conclusion, we prove it is feasible to collect *P. vivax* natural infections in the field for scRNA-seq and present the first scRNA-seq analysis of *P. vivax* blood-stage parasites from natural infections. Moreover, we show how different methods, either sequencing clinical samples directly or after ex vivo maturation, can impact the gene expression profile. Our findings represent an important step towards completing a *P. vivax* cell atlas which can be used to support future biological and translational studies.

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CONSERVED GENETIC PATHWAYS OF OXIDATIVE STRESS AND ANTIMALARIAL RESPONSES IN MALARIA PARASITE BIOLOGY

Camilla V. Pires, Chiara Micchelli, Chengqi Wang, John H. Adams

University of South Florida, Tampa, FL, United States

Oxidative stress is a central pressure shaping parasite survival during malaria infection. During the blood stage, *Plasmodium falciparum* is exposed to reactive oxygen species generated by host immune responses, febrile episodes, hemoglobin variants, and antimalarial drugs such as artemisinin. In malaria-endemic regions, hemoglobinopathies (e.g., sickle cell, thalassemia, HbE, HbC) are highly prevalent and confer strong protection against severe malaria. This protection is thought to arise partly from the pro-oxidative environment generated within hemoglobinopathic red blood cells. Also, oxidative stress is central to the mechanism of action of artemisinin drugs, the cornerstone of frontline antimalarial therapy. Thus, parasites in endemic regions experience overlapping oxidative pressures arising from both host genetics and antimalarial treatment. Despite this mechanistic and geographic overlap, how hemoglobinopathy-associated oxidative stress influences parasite adaptation and artemisinin efficacy remains poorly understood. To address this question, we used a physiologically relevant *in vitro* model of oxidized erythrocytes that mimics the redox environment of sickle red blood cells. Using this system, we observed that parasites grown under oxidative conditions display reduced sensitivity to the artemisinin derivative dihydroartemisinin, suggesting that prolonged exposure to oxidative microenvironments may predispose parasites to increased drug tolerance. To investigate the mechanisms underlying this phenomenon, we performed large-scale forward genetic screens using a *P. falciparum piggyBac* mutant library to identify genes associated with altered sensitivity to oxidative stress and dihydroartemisinin. Comparison with previous *piggyBac* screens for heat shock, and growth in sickle-trait cells revealed overlapping genetic pathways linked to lipid metabolism, exported protein functions, and RNA metabolism. Together, these findings suggest that host-derived oxidative environments reshape parasite growth dynamics and modulate antimalarial drug responses.

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STAGE-SPECIFIC REGULATION OF THE *PLASMODIUM FALCIPARUM* PROTEASOME ACTIVITY REVEALS ADAPTIVE REWIRING IN ARTEMISININ RESISTANCE

Shubha Bevkal Subramanyaswamy[1], Gourab Paul[1], Deborah Ogunribido[1], Andrea Mirauti[1], Daqiang Li[1], Wenhu Zhan[1], Gang Lin[2], Laura Kirkman[3]

[1]Weill Cornell Medicine, New York, NY, United States, [2]New York, NY, United States, [3]Weill Cornell Med College, New York, NY, United States

The ubiquitin-proteasome system (UPS) is essential for *Plasmodium falciparum* to maintain protein homeostasis, adapt to proteotoxic stress, and regulate parasite growth and stage transitions. The proteolytic 20S proteasome core is the central component of the UPS, where unfolded protein substrates are degraded into oligopeptides. Mechanisms regulating malaria parasite proteasome activity are poorly understood and have not been thoroughly studied. This knowledge gap is especially critical in the context of artemisinin (ART) resistance, where parasite survival depends on an enhanced stress response, including a greater reliance on the UPS. We profiled proteasome activity and abundance across the parasite intraerythrocytic developmental cycle (IDC) in both ART-sensitive (ART-S) Dd2 and ART-resistant (ART-R) Dd2K13^{R539T} parasites. We uncovered striking stage-specific regulation and found that ART-R Dd2K13^{R539T} parasites exhibited higher ring stage proteasome activity than ART-S Dd2 suggesting a unique adaptive rewiring of proteasome function. To study parasite proteasome regulation, we manipulated proteasome abundance in Dd2 and Dd2K13^{R539T} by creating a conditional knockdown of PfUMP1, a conserved proteasome maturation factor. These experiments uncovered two key features of proteasome regulation in *P. falciparum*: (1) the absence of canonical transcriptional upregulation in response to

decreased proteasome activity, and (2) ART-R parasites exhibit a ring-stage specific increased sensitivity to proteasome downregulation. Together, our findings reveal a previously unrecognized layer of proteasome regulation in malaria parasites and how, potentially as part of their survival adaptations to decreased hemoglobin uptake associated with ART resistance, these parasites alter proteasome function to survive. This work reinforces the therapeutic potential of the proteasome as a stage- and resistance-specific antimalarial target.

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EVALUATION OF QPCR ASSAYS FOR THE MOLECULAR IDENTIFICATION OF *DRACUNCULUS MEDINENSIS* FROM WORMS EXTRACTED FROM INFECTED HOSTS, GUINEA WORM ERADICATION PROGRAM (GWEP)

Jasmine K. Conaway[1], Margaret Neja[1], Sarah Sapp[2], Sasisekhar Bennuru[3], Michael Yabsley[4], Chrstopher A. Cleveland[5], Adam J. Weiss[6], **Vitaliano A. Cama**[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]CDC, Atlanta, GA, United States, [3]National Institutes of Health, Bethesda, MD, United States, [4]Southeastern Cooperative Wildlife Study, Univ of Georgia College of Vet Med, Athens, GA, United States, [5]Southeastern Cooperative Wildlife Disease Study, Athens, GA, United States, [6]Atlanta, GA, United States

Guinea worm disease, caused by *Dracunculus medinensis* infection, is the only parasitic disease targeted for eradication. Thus, correct and prompt parasite identification is crucial for GWEP activities. Microscopy is the diagnostic standard; however, the morphometric features of a specimen can be affected by the lengthy worm extraction process or by delayed preservation in 70% ethanol, leading to microscopy-equivocal specimens (MES). Resolution of MES is performed by DNA sequence analysis of the 18S rRNA and cox1 genes. This approach, although definitive, extends the identification process by seven or more days. To expedite parasite identification in MES, we investigated two novel qPCR assays designed to detect minute quantities of *D. medinensis* DNA: a) the cytochrome oxidase *b* gene (*cytb*), and b) the CL14 locus. DNA was obtained from 105 archived previously diagnosed specimens: 24 from microscopy positive specimens (MPS), 24 MES where *D. medinensis* was identified by DNA sequence analysis, and 57 microscopy negative specimens. Each specimen was tested in triplicate runs by each assay. For each assay, a sample was considered positive if at least two of the triplicate runs had a Ct value of ≤40; otherwise, it was considered negative. These data were used to determine the diagnostic performance characteristics of each assay. Both qPCR assays had 88% sensitivity, 100% specificity, and 94% diagnostic accuracy. The *cytb* assay missed three MPS and three MES, while the CL14 assay missed four MPS and two MES; one MES was classified negative by both assays. When the assay performance characteristics were focused on MES, the *cytb* assay had a sensitivity and diagnostic accuracy of 88% and 96%, while the CL14 assay had 92% sensitivity and 98% diagnostic accuracy. These findings show that both the *cytb* and CL14 assays can correctly identify most positive specimens, suggesting that either assay could help expedite the identification of *D. medinensis* in MES.

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NOVEL MOLECULAR DIAGNOSTICS FOR GUINEA WORM ERADICATION PROGRAM

Rachel Pietrow[1], Trinity Obiorah[1], Elizabeth Thiele[2], Jessica Van Loben Sels[3], Maryann Delea[3], Thomas B. Nutman[4], **Sasisekhar Bennuru**[1]

[1]National Institutes of Health, Bethesda, MD, United States, [2]Poughkeepsie, NY, United States, [3]Atlanta, GA, United States, [4]National Institutes of Health, Chevy Chase, MD, United States

Dracunculiasis is caused by infection with the roundworm parasite *Dracunculus medinensis* (Dm). With just 10 human cases in early 2026, dracunculiasis is on the verge of eradication despite the lack of a curative treatment or vaccine. However, the recent growth in animal reservoirs (dogs, cats, baboons) and an increased understanding of the paratenic hosts involved has complicated elimination efforts and necessitated novel diagnostics. Newly developed diagnostics are required to distinguish both pre-patent infection in animals and analytes in environmental samples, such as water and aquatic animals. Bioinformatic analysis of the sequence read archives of Dm and closely related guinea worms *D. insignis*and *D. lutrae* resulted in the identification of highly repetitive elements specific for each of the species. Screening of qPCR assays targeting multiple Dm-specific targets resulted in the selection of CL14, CL25 and CL55 that were highly sensitive to Dm genomic DNA (gDNA). Of the three targets, CL14 could detect gDNA of Dm across geographic isolates (Angola, Cameroon, Mali, South Sudan) and was reliably more sensitive than CytB and Cox3 (n=11). Additionally, CL14 was able to detect as little as a 1/10,000 dilution of a single larva following alkaline digestion (n=5). Dried blood spots spiked with 100, 10, 1 pg/µL of Dm gDNA were also detected by CL14 (n=5) after digestion, which may hold promise for field-collected samples. Preliminary experiments to detect CL14 in circulating cell-free DNA from plasma of dogs indicated no cross-reactivity with *Dirofilaria immitis* (dog heartworm; n=4), or uninfected dogs (n=2). Furthermore, CL14 was not cross reactive with closely related and unrelated nematodes. All previously confirmed field samples across geographical locations were positive for CL14. Additional testing with environmental samples from Chad highlights the potential future of CL14 as a diagnostic to monitor both animal reservoirs and environmental samples.

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SCREENING AND IDENTIFICATION OF PEPTIDES FOR ANTIBODY DETECTION TO *WUCHERERIA BANCROFTI* INFECTION

Sylvia Ossai[1], Yong Wang[1], Kimberly Y. Won[2], Sukwan Handali[1], W. Evan Secor[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Centers for Disease Control & Prevention, Atlanta, GA, United States

Following validation of elimination of lymphatic filariasis (LF), countries are required to implement post-validation surveillance to monitor potential resurgence and ensure the sustained interruption of transmission. Antibody detection offers a sensitive method for identifying early exposure and may provide advantages over antigen detection for detecting recrudescence. However, many available antigens such as Wb123 and WbSXP-2 used in LF serology demonstrate cross-reactivity with other filarial parasites, including *Onchocerca volvulus, Mansonella perstans,* and *Loa loa.* To address this limitation, we performed *in silico* prediction of linear B-cell epitopes from *Wuchereria bancrofti* proteome and identified 120 candidate peptides. In parallel, we analyzed 14 available antigens and identified 29 candidate peptides using bioinformatics tools, including B-cell epitope prediction and antigenicity filtering. All selected peptides were synthesized as biotinylated constructs and evaluated using peptide ELISA to identify markers capable of detecting *W. bancrofti* infection across 3 endemic regions (Haiti, Kenya, and Papua New Guinea) without cross-reactivity. We chose stringent criteria for peptide selection (optical density (OD) values >1 and signal-to-noise ratio >5) that were not met by any single peptide for sera from all 3 endemic regions. However, 61 peptides demonstrated OD >1 in at least one endemic region without detectable cross-reactivity and were not recognized by sera from persons with *Onchocerca volvulus, Mansonella perstans,* or *Loa loa* infections. These candidates will be further evaluated and combined to generate chimeric proteins designed to achieve broader geographic reactivity while enhancing specificity and sensitivity for LF serological surveillance.

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PATIENT-REPORTED OUTCOME MEASURES AS PROOF OF TREATMENT EFFICACY IN OCCULT LOIASIS: A RANDOMIZED CONTROLLED PROOF OF CONCEPT TRIAL

Saskia D. Davi[1], Johannes Mischlinger[1], Lillian R. Endamne[1], Lars Matthies[1], Ghyslain Mombo-Ngoma[2], Tanja Stamm[3], Valentin Ritzel[3], Cédric Mbavu[1], **Pia M. Michelitsch**[1], Michael Ramharter[1], Rella Zoleko-Manego[2]

[1]Bernhard-Nocht-Institut für Tropenmedizin, Center for Tropical Medicine Bernhard Nocht Institute for Tropical Medicine & I Dept. of Medicine University Medical Center Hamburg-Eppendorf, Hamburg, Germany, [2]Centre de Recherches Medicales de Lambarene, Lambarene, Gabon, [3]Section for Outcomes Research, Center for Medical Statistics, Informatics, and Intelligent Systems, Vienna, Austria

Loiasis, a vector-borne chronic infection caused by *Loa loa* and transmitted by *Chrysops* spp., is a tropical disease affecting millions in West and Central Africa. While research has mainly focused on microfilaraemic loiasis due to the risk of severe adverse events following antifilarial treatment with ivermectin (IVM), individuals with occult loiasis remain largely neglected in research and therapeutic interventions. Because parasitological endpoints are often absent in occult loiasis, treatment success and effectiveness are difficult to assess. Patient-reported outcome measures (PROMs) may offer a valuable alternative to capture disease burden and treatment response. We conducted a randomised, controlled, open-label proof-of-concept trial in Gabon between May 2023 and June 2025. Adults with occult loiasis were randomised to receive IVM (single dose of 200 µg/kg), albendazole (ALB) (400 mg daily for 28 days), or placebo (PLA). A disease-specific PROM instrument was developed to assess symptom burden, limitations in daily activities, and perceived health. PROMs were collected longitudinally from baseline to day 180. Intra-group changes were analysed using the Friedman test, and between-group comparisons using the Kruskal-Wallis test. Among 93 screened individuals, 58 participants (58/93, 52%) were eligible for inclusion, with a mean age of 45 years (SD = 12); 78% (45/58) were female. Nineteen participants were enrolled in the ALB arm, 20 in the IVM arm, and 19 in the PLA arm. At baseline, PROM scores did not differ significantly between groups. Pruritus and fatigue were frequently reported, with mostly mild severity. The greatest reduction in symptom burden was observed at day 7. The ALB arm demonstrated the highest number of significant intra-group PROM reductions, followed by the IVM arm, while the PLA arm showed fewer changes. This study is the first to apply PROMs as a primary endpoint to assess treatment effectiveness in occult loiasis. The findings suggest that PROMs are a feasible and informative tool for monitoring treatment response in this neglected population, particularly where parasitological endpoints are lacking.

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DETAILED CHARACTERIZATION OF A NEW RAPID TEST FOR ONCHOCERCIASIS MAPPING PROGRAMS

Daniela Oltean[1], Sam Marton[1], Justin Nueve[2], KathyJean Farnam[3], Kimberly Y. Won[4], Scott Elder[5], Abakar Gankpala[6], Kerstin Fischer[7], Peter Fischer[8], Jenna E. Coalson[9], Chukwuemeka Cletus Makata[10], Martins Imhansoloeva[11], Sasisekhar Bennuru[12], Yvonne Ashong[13], Dziedzom Komi de Souza[14], **Marco A. Biamonte**[2]

[1]San Diego, CA, United States, [2]Big Eye Diagnostics, San Diego, CA, United States, [3]Drugs & Diagnostics for Tropical Diseases, San Diego, CA, United States, [4]Centers for Disease Control & Prevention, Atlanta, GA, United States, [5]Centers for Disease Control and Prevention, Atlanta, GA, United States, [6]Liberian Institute for Biomedical Research, Saint Louis, MO, United States, [7]Washington University School of Medicine, Saint Louis, MO, United States, [8]Washington University School of Medicine, St. Louis, MO, United States, [9]The Carter Center, Atlanta, GA, United States, [10]Federal Ministry Of Health, Nigeria, Abuja, FC, Nigeria, [11]Sightsavers, Kensington, Australia, [12]National Institutes of Health, Bethesda, MD, United States, [13]Accra, AA, Ghana, [14]Noguchi Memorial Institute for Medical Research, Legon, Ghana

In 2021, the World Health Organization (WHO) published a Target Product Profile (TPP) for tests intended to support the mapping of onchocerciasis in areas of low endemicity. The TPP requires relatively low sensitivity (≥60%) but high specificity (≥99.8%). We have developed a rapid biplex test that detects human IgG4 antibodies to Ov16 on one line, and to a combination of Ov33 and OvOC-3261 on the second line. To prioritize high specificity, both test lines must be visible for a positive test result. The test is intended for use with fresh capillary blood or dried blood spots (DBS). Manufacturing procedures have been developed and demonstrated on pilot production lots ranging from 5,000-40,000 tests which form the backbone to a regulatory dossier to be approved by WHO. In independent validations, the sensitivity with serum samples and heparinized whole blood samples was 83.1% [95% CI 76.3-92.2; n=72, CDC] and 75.5% [70.7-79.8; n=335, Liberia], respectively, thus meeting the TPP requirement of ≥60% even at the lowest bound of the 95% confidence intervals. A field evaluation using capillary blood in three Nigerian states post-MDA revealed 12 positives among the 1913 children and adolescents tested, leading to a minimum specificity of 99.4% [98.9-99.6; n= 1913]. In sera, specificity against other infections, including *Wuchereria bancrofti*, was 100% [97.5-100; n=158, CDC], and against *Mansonella perstans* samples was ≥97.5% [87.1-99.9; n=40, NIH]. In-house, presumed uninfected USA sera mixed with red blood cells showed a specificity of 99.0% [97.7-99.6; n=497]. Matrix equivalence studies in Ghana and Liberia showed strong clinical agreement between whole blood, DBS, and EDTA plasma, with kappa = 0.826-0.921, depending on the comparison. Stability studies with the formal pilot lots are ongoing, while an engineering lot proved stable at 40 °C for ≥ 18 months and at 50 °C for 7 months. In Nigeria, the rate of invalids was 12/13,551 (0.26%). The 99.0% specificity in North American samples is below the TPP requirement and will need re-investigation, while under field conditions, the specificity is higher (99.4%). The device meets all other TPP requirements.

7297

EVALUATION OF RECONSTITUTED LYOPHILIZED OV16 ANTIGEN STORAGE METHODOLOGIES TO IMPROVE ONCHOCERCIASIS DIAGNOSTICS

Emma C. Underwood[1], Camila Trejo Paz[2], Hassan Hassan[1], Thomas R. Unnasch[2], Kristi Miley[1]

[1]University of South Florida, Tampa, FL, United States, [2]Tampa, FL, United States

The World Health Organization's guidelines regarding onchocerciasis elimination advise measuring disease prevalence in children to assess transmission and suppression. These guidelines recommend implementation of serological testing using Enzyme-Linked Immunosorbent Assay (ELISA) with an *Onchocerciasis volvulus* antigen specific antigen, Ov16. The Ov16 ELISA requires purified Ov16 recombinant antigen has historically required a cold chain, something that can be difficult to maintain when shipping internationally. To overcome this problem, we have explored the stability of the Ov16 antigen after being reconstituted and stored either at room temperature or at 4°C. Plates were coated with the reconstituted antigen from both storage conditions in duplicates weekly for 12 weeks and tested using standard ELISA protocols with monoclonal antibody standards and known positive dried blood spots. A plate coated with fresh antigen was conducted in parallel as a control. Preliminary results showed reconstituted antigen performed as well as a plate with freshly coated antigen up to 63 days. This shows the efficacy of storing non-lyophilized antigen for international shipping, eliminating the need for a cold chain and improving throughput of diagnostic assays in endemic regions.

7298

ANTI-COLLAGEN ANTIBODIES AS BIOMARKERS FOR *ONCHOCERCA VOLVULUS* INFECTION

Sarah E. Greene, Yuefang Huang, Peter Fischer
Washington University School of Medicine, Saint Louis, MO, United States

Onchocerciasis, or river blindness, is a neglected tropical disease caused by the filarial nematode *Onchocerca volvulus* that affects millions of people in sub-Saharan Africa. The World Health Organization has targeted onchocerciasis for elimination and prioritized the production of improved diagnostic tests to support this goal. We have previously used proteomics and laser capture microdissection to identify targets found only in the intra-uterine microfilariae of *O. volvulus* worms and not in other worm tissues or filarial species. With this approach, we identified the target OVOC12404 and an ELISA based on quantification of IgG to this target was sensitive and specific for onchocerciasis. Interestingly, OVOC12404 is a member of a family of mini-chain collagens. Here, we characterized 3 closely related collagen targets, OVOC12544 OVOC12587 and OVOC12597, to assess if they could enhance a diagnostic assay based on OVOC12404. Identification of IgG antibodies to OVOC12597 had low sensitivity. However, onchocerciasis patients did produce antibodies to both OVOC12544 and OVOC12587. Using ELISA, we detected IgG antibodies to OVOC12544 and OVOC12587 in plasma from 54% and 51% of people with *O. volvulus* microfiladermia, respectively. While less sensitive than the ELISA for OVOC12404, these new targets are interesting as they could increase assay sensitivity when combined with OVOC12404. Based on *in silico* analysis, a combination assay using OVOC12404 and OVOC12544 should identify 100 of 123 (81%) of onchocerciasis patients, while a combination assay using OVOC12587 should identify 121 of 147 (82%) of onchocerciasis patients. An important feature of a diagnostic based on OVOC12404 is that antibody levels decline significantly after treatment. This is also true of the IgG responses to OVOC12544 and OVOC12587. Importantly, initial testing of these new targets demonstrated 100% specificity for *Wuchereria bancrofti* and non-endemic control samples. Further work is needed to assess the value of collagen-based antibody tests as additional diagnostic tools to complement other antibody tests and support onchocerciasis elimination programs.

7299

POOL SIZE LIMITS FOR THE DETECTION OF *ONCHOCERCA VOLVULUS* DNA IN WHOLE *SIMULIUM* BLACK FLIES USING ROUTINE PROTOCOLS

Kerstin Fischer[1], Nils Pilotte[2], Matthew R. Kulpa[3], Megan E. Fris[4], Peter U. Fischer[5]
[1]Washington University School of Medicine, Saint Louis, MO, United States, [2]Quinnipiac University, Northampton, MA, United States, [3]Saint Louis, MO, United States, [4]Quinnipiac University, Hamden, CT, United States, [5]Washington University School of Medicine, St. Louis, MO, United States

Onchocerca volvulus is a leading cause of skin and eye disease that infects ~ 20 million people in Africa. Mass drug administration (MDA) with ivermectin has been used to control/eliminate the disease for over 40 years with increasing success. To verify interruption of transmission and for post elimination surveillance accurate and robust diagnostic tools are needed. Molecular xenomonitoring (MX), using *Simulium* blackflies to detect *O. volvulus* in a population has some advantages compared to surveys in humans. When only *Simulium* heads are pooled, DNA of infective *O. volvulus* larvae (L3) is detected as an indicator of transmission, while when entire blackflies are pooled, DNA of dead and developing larvae is detected regardless of the ability to transmit *O. volvulus*. Therefore, MX of whole blackflies is a sensitive indicator of the presence of *O. volvulus* in the human population. To optimize the use of whole blackflies it is essential to identify pooling limits that prevent DNA detection. Initial processing of blackfly pools was done in duplicated increments of 20 black flies (i.e., 20, 40, 60, 80, 100) with two spiked *O. volvulus* microfilariae per pool. Extracted material was amplified by qPCR with primers targeting the OvND5 gene or the O150 repeat. An unrelated DNA sequence was added to all pools as an efficiency control and was detected by qPCR in parallel. Regardless of pool size, Ct values averaged 33.91 (31.76 - 35.50) for OvND5 and 35.63 (33.87 - 37.24) for O150. The same technique was then repeated with 10 pools of 100 black flies. Ct values for OvND5 averaged 33.93 (31.93 - 34.81) and O150 had detectable Ct values in 8/10 samples (n=8; 30.70 (27.06 - 35.96)). Results have been confirmed by an independent laboratory and by using different thermocyclers. Overall, detection of *O. volvulus* DNA was not limited by increasing pool sizes of up to 100 black flies and performed more reliably using OvND5 primers. However, additional buffer is required for larger pool sizes (i.e., 80-100) due to liquid absorption, but this did not affect the detection limit. MX using whole blackflies has the potential to improve validation and surveillance of *O. volvulus* elimination.

7300

MOXIDECTIN COMBINATION TREATMENTS CLEAR *ONCHOCERCA VOLVULUS* SKIN MICROFILARIAE MORE EFFICIENTLY THAN IVERMECTIN COMBINATIONS

Peter Uwe Fischer[1], Lincoln S. Gankpala[2], Cooper W. Sannah[2], Aaron Momolu[2], Patrick Kpanyen[2], Kerstin Fischer[3], Alexandre Dyer[3], Gary J. Weil[3]
[1]Washington University School of Medicine, St. Louis, MO, United States, [2]National Public Health Institute of Liberia, Monrovia, Liberia, [3]Washington University School of Medicine, Saint Louis, MO, United States

Onchocerciasis (river blindness) elimination programs would benefit from treatment regimens that kill or permanently sterilize adult worms. In a randomized, observer masked, hospital-based clinical trial we are comparing two doses of ivermectin or moxidectin (Mox) combination treatments given 6 months apart. Participants (all microfilaria, Mf, positive) were pretreated with ivermectin 6 months before randomization to one of four experimental treatments. So far, we followed up 160 of 279 study participants one year after two rounds of experimental treatment. Study arms included standard doses of ivermectin plus albendazole (IA), IA plus diethylcarbamazine (IDA), Mox plus albendazole (MoxA) and MoxA plus diethylcarbamazine (MoxDA). Skin Mf were not detected in snips taken 6 months after the first experimental treatment with IA, IDA, MoxA or MoxDA in 81%, 81%, 92%, and 89% of study participants, respectively. Twelve months after the second experimental treatment, skin snips were free of Mf in 62%, 50%, 74% and 85% of individuals in each arm. At that time point mean Mf densities in Mf positive individuals were below 1 Mf per mg skin in all groups. Not unexpectantly, the two moxidectin combination treatments 6 months apart lead to longer lasting clearance of Mf compared to the two ivermectin combination treatments 6 months apart (P=0.0055). No significant differences in Mf clearance were seen between IA and IDA or MoxA and MoxDA. These preliminary results may also indicate differences in adult worm viability since these worms produce Mf. The primary study outcomes are skin Mf clearance and female worm fertility and will be assessed by nodulectomy 18 months after the second dose of experimental treatment, but the current interim results are promising. Short-term oral treatment that is superior to ivermectin or IA for achieving sustained Mf clearance or sterilization of adult worms could significantly accelerate onchocerciasis elimination in Africa.

7301

INVESTIGATING THE DIVERSITY AND PREVALENCE OF NATURAL *WOLBACHIA* STRAINS IN *ANOPHELES* MALARIA VECTORS THROUGH MULTIPLE DETECTION METHODS

Isabel Hughes[1], Izie Lopez-Scott[1], Jade Ball[1], Elin Cunningham[1], Seynabou Sougoufara[2], Laura Chatterley[1], Janvier Bandibabone[3], Cyprian Adala[4], Shehu Shagari Awandu[5], Eva Heinz[6], Erin Gorsich[1], Thomas Walker[1]
[1]The University of Warwick, Coventry, United Kingdom, [2]Coventry, United Kingdom, [3]Centre de Recherche en Sciences Naturelles de Lwiro, Lwiro, Democratic Republic of the Congo, [4]Kenya Medical Research Institute-

Center for Global Health Research, kisumu, Kenya, [5]Jaramogi Oginga Odinga University of Science and Technology, Kisumu, Kenya, [6]University of Strathclyde, Glasgow, United Kingdom

Malaria is a life-threatening disease caused by *Plasmodium* protozoa and spread by *Anopheles* mosquitoes, with an estimated 282 million global cases in 2024. New vector control strategies are required due to rising insecticide resistance and the spread of the invasive urban species *Anopheles stephensi* across the WHO Africa Region. *Wolbachia* is an endosymbiotic bacterium that can invade mosquito populations and inhibit human pathogens. Its success in reducing dengue incidence through releases of *Aedes* mosquitoes is driven in part by cytoplasmic incompatibility, a reproductive phenotype that enhances spread through host populations. In *Anopheles*, *Wolbachia* has been shown to inhibit *Plasmodium* development, and stable transinfections in *An. stephensi* have been generated. However, further progress for malaria biocontrol requires a greater understanding of *Wolbachia* diversity and prevalence in natural *Anopheles* populations. To date, genuine *Wolbachia* endosymbiosis has been documented in only two *Anopheles* species, *An. demeilloni* and *An. moucheti.* However, PCR-based studies have reported low and variable *Wolbachia* prevalence across collection sites. *Wolbachia* prevalence rates in *An. demeilloni* were 37.8% in Kenya and 89.3% in the Democratic Republic of Congo respectively. These discrepancies may reflect methodological limitations, including reduced sensitivity at low bacterial densities and challenges in distinguishing morphologically similar species. This highlights the need to distinguish true biological prevalence from technical bias. This study optimises DNA extraction methods and develops two qPCR-based detection assays for *Wolbachia* in *Anopheles*, while assessing compatibility with downstream Illumina and Nanopore sequencing. Additionally, tissue-specific microbiome composition and *Wolbachia* strain diversity are characterised to support the discovery and sequencing of novel strains. These findings aim to improve detection accuracy and inform strain selection strategies for *Wolbachia*-based malaria biocontrol.

7302

LANDSCAPE AND CLIMATIC DRIVERS OF RURAL DENGUE AND *AEDES* MOSQUITO DISTRIBUTION IN COSTA RICA: COMBINED MODELING AND FIELD SURVEYS

Talya Shragai[1], Caroline Glidden[1], Emma Krasovich Southworth[1], Kota Osawa[2], Luis Enrique Chaves-Gonzalez[3], Diana Rojas Araya[2], Rodrigo Marin[4], Adriana Troyo[5], Erin Mordecai[1], **Rachel Fay**[1]

[1]Stanford University, Stanford, CA, United States, [2]University of Costa Rica, San Jose, Costa Rica, [3]University of Costa RIca, San Jose, Costa Rica, [4]Ministry of Health, Costa Rica, San Jose, Costa Rica, [5]Universidad de Costa Rica, San Jose, Costa Rica

Dengue is increasingly recognized as a rural public health burden, yet the ecological drivers of rural transmission remain poorly understood. We took a two-pronged approach to investigate landscape and climatic drivers of rural dengue in Costa Rica. First, we fit a Bayesian spatio-temporal model to 10 years of district-level case data to identify the climatic and land-use features associated with dengue risk. Second, we conducted entomological field surveys across 100 sites spanning residential, agricultural, and forest-edge environments in two rural dengue-endemic regions to assess how land use shapes the distribution of each species. Modeling results showed that dengue occurs in many rural environments primarily because they are located at lower elevations where temperatures are suitable. Modest levels of built infrastructure substantially increased dengue risk - risk increased by over 30% between districts at minimum building volume and those with 500,000m^3 more. However, further increases beyond the mean were associated with only a 0.25-3% rise, suggesting that a low threshold is needed to sustain transmission. Dengue risk was positively associated with agricultural crop cover at low and mid elevations but not at high elevations (low elevation RR = 1.39, 95% CI: 1.16-1.66; mid elevation RR = 1.94, 95% CI: 1.61-2.33). Field surveys identified both *Aedes aegypti* and *Ae. albopictus* as widespread. Ae. aegypti abundance increased sharply with built cover and temperature (both $p < 0.001$), while Ae. albopictus showed a more gradual response across the built cover gradient ($p = 0.033$) and peaked at cooler, more vegetated settings. Together, these results suggest that rural dengue burden in Costa Rica reflects climate suitability aligning with low thresholds of built infrastructure, with agriculture likely operating indirectly rather than through direct larval production in crop environments. Given the large populations living just above the current elevational limit for climate suitability, warming temperatures could shift dengue transmission into more populated areas.

7303

EFFICACY OF AN ATTRACTIVE LETHAL OVITRAP TO REDUCE POPULATIONS OF *AEDES* MOSQUITOES: A CONTROLLED TRIAL IN IQUITOS, PERU

Dawn M. Wesson[1], Valerie Andrea Paz-Soldan[2], Kanya Claudine Long[3], Loganathan Ponnusamy[4], **Samuel Jameson**[1], Robin M. Moudy[1], Justin K. Davis[1], Helvio Astete Vega[5], Isabel Bazan Arista[5], Stalin Vilcarromero[6], Siles Crystyan[5], Carolina L. Guevara[7], Eric S. Halsey[8], Coby Schal[4], Thomas W. Scott[9], Charles S. Apperson[10], Amy C. Morrison[11]

[1]Tulane University, New Orleans, LA, United States, [2]Tulane University School of Public Health & Tropical Medicine, Lima, Peru, [3]University of California, San Diego, La Jolla, CA, United States, [4]North Carolina State Univ, Raleigh, NC, United States, [5]Namru-6, Iquitos Punchana, Peru, [6]US NAMRU-6, Iquitos, Peru, [7]US Naval Medical Reseaarch Unit No 6, Lima, Peru, [8]Centers for Disease Control and Prevention, Atlanta, GA 30305, United States, [9]Univ of California Davis, Luck, WI, United States, [10]North Carolina State University, Raleigh, NC, United States, [11]University of California Davis, Davis, CA, United States

Dengue remains the most important arboviral infection worldwide, transmitted primarily by *Aedes aegypti* mosquitoes closely associated with human habitations and household containers. Vector control is the principal prevention strategy, but novel tools targeting the epidemiologically relevant gravid female are urgently needed. We evaluated the Attractive Lethal OviTrap (ALOT), a device combining a larvicide, insecticide-treated material, and bacterial oviposition attractant designed to attract and kill gravid females, for public health impact against dengue in a proof-of-concept field efficacy trial in Iquitos, Peru. After three baseline entomological surveys, 5,280 ALOTs were placed in core (n=753 houses) and buffer (n=1,598 houses) areas at 85% household coverage, alongside a no-trap control area (n=1,233 houses). Entomological surveys were conducted every two months and 7,492 residents were monitored for dengue disease three times per week over 3.5 years. Adult mosquito abundance was analyzed using negative binomial generalized additive mixed models; time to first dengue infection was compared using Kaplan-Meier analysis and restricted mean time lost methods. Total *Ae. aegypti* abundance was initially reduced by 52% (RR=0.48, 95% CI: 0.40-0.58, $p<0.001$) and female *Ae. aegypti* by 47% (RR=0.53, 95% CI: 0.43-0.66, $p<0.001$), though both effects diminished over the study period. Non-*Ae. aegypti* species showed a similar initial reduction. Cumulative dengue incidence over three years was 1.40% (95% CI: 1.00%-1.70%) in the ALOT area versus 3.20% (95% CI: 2.60%-3.80%) in the control area, a 56% relative reduction (log-rank $\chi^2=30.0$, $p<0.001$). Participants in the intervention area remained dengue-free 15.5 additional days on average (95% CI: 11.1-20.0, $p<0.001$). The ALOT strategy decreased but did not eliminate female vector densities yet demonstrated clear public health impact, reducing dengue disease burden. These results support further evaluation of lethal ovitraps as a component of integrated dengue control, while underscoring implementation challenges that must be addressed before large-scale deployment.

7304

PERCEPTION OF ATTRACTIVE TARGETED SUGAR BAITS, A NOVEL MALARIA CONTROL INTERVENTION, BY ADOLESCENT CHILDREN FROM WESTERN KENYA

Caroline Ogwang[1], Teresa Bange[1], Dominic Ouma[1], Corrie Omollo[1], Brian Seda[1], Julie R. Gutman[2], Jonathan Schultz[3], Simon Kariuki[1], Feiko ter Kuile[4], Sarah G. Staedke[4], George Okello[1]

[1]KEMRI/Centre for Global Health Research, Kisumu, Kenya, [2]Centers for Disease Control Prevention, Atlanta, GA, United States, [3]Saint Alphonsus, Boise, ID, United States, [4]Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Attractive Targeted Sugar Baits (ATSBs) are a novel malaria control intervention that aim to attract and kill mosquitoes. Understanding factors influencing community acceptability of ATSBs is important for guiding future implementation. We conducted a qualitative evaluation of perceptions of ATSBs by adolescent children in the setting of an ongoing cluster-randomised trial in western Kenya evaluating ATSB impact on malaria burden. Twelve focus group discussions (FGDs) were conducted each with a purposive sample of 8-12 children aged 13-17 years; six from clusters with a high number of ATSB removal cases, and six with a few cases of ATSB removal. Following parental consent and participant assent, FGDs were conducted in schools, recorded, transcribed, and imported into Nvivo 12 for coding and analysis. A thematic approach to data analysis was adopted. 60 boys and 64 girls were interviewed. Children received information about ATSBs from study staff and their parents/guardians. Most understood that ATSBs contained an insecticide and were designed to kill mosquitoes and control malaria. Despite warnings, some children from 11 out of 12 FGDs reported touching ATSBs to examine their contents and to better understand how they worked while others did not since they were warned by parents/guardians or study staff. Some children reported having prevented younger children from touching ATSBs and alerted their parents about dislodged ATSBs. With respect to perceived efficacy, some children noted ATSBs reduced mosquitoes and malaria cases and explained that they could read at night without mosquitoes biting them. Others noted little or no change, or even a perceived increase, in mosquito numbers. Children believed that engaging them directly was important for successful ATSB implementation and suggested they could champion ATSBs. School-aged children are curious and can act as change agents for their communities. Engaging children in the implementation of vector control tools could promote their safety and community acceptance.

7305

EVALUATING MALARIA VECTOR CONTROL THROUGH POPULATION PHARMACOKINETIC AND PHARMACODYNAMIC MODELING AND SIMULATION OF IVERMECTIN

Katherine Kay[1], Frederic Wendlamita Nikiema[2], Megan C. Pane[3], Greg Pugh[4], Da Ollo[5], Emma J. Wu[6], Cheick Oumar Wendpagnandé Ouedraogo[2], Sie R. Dah[5], Emmanuel Sougue[5], Brian D. Foy[4], Roch Dabire[5], Riggs Matthew[3], A. Fabrice SOME[5], **Sunil Parikh**[7]

[1]Metrum Research Group, [2]Institut de Recherche en Sciences de la Sante, Bobo Dioulasso, Burkina Faso, [3]Metrum Research Group, Tarriffville, CT, United States, [4]Colorado State University, Fort Collins, CO, United States, [5]Institut de Recherche en Sciences de la Sante, Bobo-Dioulasso, Burkina Faso, [6]Colorado School of Public Health, Aurora, CO, United States, [7]Yale School of Public Health, New Haven, CT, United States

The RIMDAMAL II trial assessed monthly ivermectin (IVM) mass drug administration (MDA) as a malaria vector control tool in Burkina Faso. Population pharmacokinetic and pharmacodynamic modeling and simulation was conducted using venous IVM concentrations over 28 days from 97 participants receiving 300 mcg/kg once daily x 3 days (QDx3) across adults (>19 yrs, n=40), adolescents (≥11 to 19 yrs, n=28), and children (6 to <11 yrs, n=29). The final two-compartment model, with first-order oral absorption and elimination, included an estimated effect of age group (<19 years) on oral clearance (CL/F). In adolescents and children, IVM CL/F was estimated to be 286% (95% CI 242% to 338%) of the adult CL/F, corresponding to more rapid elimination and lower exposure. IVM exposure and the influence of age, dosing regimen, and drug sensitivity on the probability of exceeding and the duration above mosquitocidal targets (LC50s) were simulated, and compared per-protocol height-based dosing targeting 300 mcg/kg QDx3 to 600 mcg/kg QDx3, and 400 mcg/kg once. Subject-level simulations showed a single 400 mcg/kg dose produced Cmax values similar to the 300 mcg/kg QDx3 but with substantially lower total exposure. Population-level simulations assessed IVM concentrations relative to LC50 thresholds from 4 to 64 ng/mL in A. gambiae. Although adults consistently remained above LC50 thresholds for longer than in adolescents and children (e.g., under 300 mcg/kg QDx3 and LC50 = 16 ng/mL, by day 7, only ~70% of adults had IVM levels above LC50, declining to ~8% by day 14, and <1% in pediatric groups on days 7 and 14), total duration above LC50 in all age groups was short. All regimens indicated adolescents and children had lower exposure than adults, which was mechanistically consistent with the model-estimated reduction in CL/F. Attaining target mosquitocidal IVM concentrations was strongly dependent on dose, age group, and Anopheles drug sensitivity. Sustaining target concentrations beyond 7 days appeared to be challenging using the doses deployed in recently completed IVM MDA trials, particularly in pediatric populations across all evaluated regimens.

7306

DEPLOYMENT STRATEGIES FOR NEXT-GENERATION MIXTURE INSECTICIDE-TREATED NETS TO REDUCE MALARIA BURDEN: A MODELLING STUDY

Thiery Masserey[1], Clara Champagne[2], Neil Hobbs[3], Swapnoleena Sen[1], Thomas A. Smith[4], Nakul Chitnis[5]

[1]Swiss Tropical and Public Health Institute, Allschwil, Switzerland, [2]Swiss TPH, Allschwil, BL, Switzerland, [3]London School of Hygiene and Tropical Medicine, London, United Kingdom, [4]University of Basel, Swiss Tropical & Public Health Institute, Basel, Switzerland, [5]Swiss Tropical & Public Health Institute, Allschwil, BL, Switzerland

Insecticide-treated nets (ITNs) are a cornerstone of malaria prevention. Increasing insecticide resistance has reduced the effectiveness of pyrethroid-only (PYR) ITNs. Next-generation mixture (NGM) ITNs, combining two insecticides, offer higher protection but at a higher procurement cost. Consequently, delivering NGM-ITNs will require greater financial investment, which is unlikely under current funding constraints. Options to mitigate their higher cost include distributing fewer nets per campaign or lowering their distribution frequency. While neither option is ideal, these are pragmatic choices that must be considered under budget constraints. However, it remains unknown how implementing more effective NGM-ITNs at lower coverage or frequency would affect malaria cases. Using an individual-based model of malaria transmission (OpenMalaria), we compared the impact on malaria cases of deploying PYR-ITNs versus deploying NGM-ITNs at reduced coverage (25% or 50%) or reduced deployment frequency (every 4 or 6 years instead of 3) in settings with PYR-resistance. ITN effects were parameterized based on entomological studies and validated against clinical trials. While deploying NGM-ITNs at high coverage every three years offered the greatest malaria protection, our findings suggest NGM-ITNs would outperform PYR-ITNs even if they must be deployed with a 25% reduction in coverage or distribution frequency due to funding constraints. Reducing deployment frequency was more effective when NGM-ITNs maintained protection between distribution campaigns. In contrast, when ITN's retention was short, reducing coverage was more advantageous as it prevented malaria rebound between distribution campaigns. Under the reduced-coverage scenario, individuals who did not access an ITN (because of the reduced coverage) still benefited from the switch to NGM-ITNs, due to stronger community protection. Thus, replacing PYR-ITNs with NGM-ITNs, even if they must be deployed at lower coverage or deployment frequency due to budget constraints, would provide better protection for the whole population in settings with PYR resistance.

7307

INSECTICIDE CHOICE, COVERAGE VARIATION, AND MALARIA OUTCOMES ASSOCIATED WITH INDOOR RESIDUAL SPRAYING IN A MESOENDEMIC BORDER AREA OF ZIMBABWE

Kayla Seggelke[1], Veronika R. Laird[2], Sungano Mharakurwa[3], Shungu Munyati[3], Lovemore Gwanzura[3], Lisa R. Yanek[3], Timothy Shields[4], Tamaki Kobayashi[4], Douglas E. Norris[4], William J. Moss[1], Matthew M. Ippolito[5]

[1]Department of International Health, Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD, United States, [2]Johns Hopkins University School of Medicine, Baltimore, MD, United States, [3]Biomedical Research Training Institute, Harare, Zimbabwe, [4]Johns Hopkins Malaria Research Institute, W. Harry Feinstone Department of Molecular Microbiology and Immunology, Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD, United States, [5]Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, United States

Indoor residual spraying (IRS) is a key malaria control intervention, yet comparative epidemiologic evidence on the population-level impact of different insecticide classes remains limited, particularly in areas with pyrethroid resistance. We evaluated associations between IRS insecticide class and malaria burden using longitudinal surveillance data from Mutasa District, Zimbabwe (2012–2020), a mesoendemic region with sustained transmission on the border with Mozambique. The analysis included 239,279 health facility malaria cases and community surveillance of 2,609 individuals in sprayed households across sequential IRS campaigns using pyrethroids, pirimiphos-methyl, and DDT. Adjusted negative binomial and generalized additive mixed models were used to estimate associations between IRS type, community-wide coverage, and malaria outcomes, accounting for demographic, spatial, seasonal, and climatological factors. Both pirimiphos-methyl and DDT were associated with lower malaria burden compared to pyrethroids across multiple epidemiologic indicators. At the facility level, DDT was associated with 65.7% lower malaria incidence than pyrethroids (95% CI: 60.4–70.2%) and 19.9% lower than pirimiphos-methyl (95% CI: 4.2–33.0%), while pirimiphos-methyl was associated with a 57.1% reduction versus pyrethroids (95% CI: 50.5–62.8%). Consistent reductions were observed for infection and clinical disease at the individual level, with DDT showing the largest estimated associations. Higher IRS coverage was independently associated with lower malaria burden, with each 10-percentage-point increase associated with a 14.0% reduction in incidence (95% CI: 9.5–18.3%, P<0.0001). Notably, DDT-based IRS was associated with lower malaria burden despite coverage levels below commonly targeted thresholds, suggesting that optimal coverage targets may vary by insecticide and setting. These findings provide epidemiologic evidence that both insecticide selection and coverage are important factors associated with IRS performance and support context-specific strategies for insecticide rotation and implementation.

7308

PHYSICAL AND CHEMICAL DURABILITY OF A PYRETHROID- PIPERONYL BUTOXIDE NET DISTRIBUTED IN ONDO AND ANAMBRA STATES OF NIGERIA

Saliu Ogunmola[1], Abiola Oluwaseun Oluwagbemiga[2], Azuka Iwegbu[3], Ezihe Chukwuebuka[4], Tarekegn A. Abeku[5]

[1]Malaria Consortium Nigeria, Abuja, Nigeria, [2]Abuja, Nigeria, [3]Malaria Consortium Nigeria, Akure South, ON, Nigeria, [4]Malaria Consortium, Abuja, Nigeria, [5]Malaria Consortium, London, United Kingdom

Insecticide-treated nets (ITNs) incorporating piperonyl butoxide (PBO) have demonstrated enhanced effectiveness in areas with pyrethroid resistance. However, evidence on their long-term physical and chemical durability under field conditions remains limited. This study assessed the durability of pyrethroid-PBO ITNs over 36 months in Ondo and Anambra States, Nigeria.A multistage stratified cluster sampling design was used to select households from 52 clusters in Ondo and 24 in Anambra, 3–4 months after campaigns distributing ITNs containing alpha-cypermethrin and PBO. A prospective cohort study was conducted in selected households in both states. Cohort nets were assessed at baseline and at 12, 24, and 36 months post-campaign. Physical integrity was evaluated using the proportionate hole index (pHI), while alpha-cypermethrin and PBO contents were measured through high-performance liquid chromatography.The proportion of serviceable nets declined steadily in both states. In Ondo, serviceable nets declined to 99.3% at 24 months and 93.5% at 36 months among nets remaining in households. In Anambra, the corresponding proportions were 100% at 24 months and 98.5% at 36 months. However, attrition due to wear and tear at 36 months reached 43.3% in Ondo and 16.7% in Anambra. Frequent washing, rough handling and environmental factors contributed to net deterioration.Chemical analysis of ITN samples from Ondo showed a 17.3% reduction in alpha-cypermethrin content (5.2g/kg to 4.3g/kg) and a 56.3% reduction in PBO (1.6g/kg to 0.7g/kg) by 36 months compared with baseline. Alpha-cypermethrin fell below acceptable level at 24 months, while PBO dropped below acceptable level at 12 months.The study demonstrated that distributed nets maintained good physical durability over the three years, but PBO content declined substantially faster than the pyrethroid component. These findings highlight the importance of strengthening community education on net care to prolong functional life. The rapid loss of PBO also indicate the need to consider ITNs incorporating dual active ingredients in areas affected by pyrethroid resistance.

7309

MOSQUITO SALIVARY PROTEIN ANTIBODIES AS EFFICIENT ENDPOINTS FOR VECTOR CONTROL TRIALS: A MECHANISTIC COMPARISON WITH CLINICAL AND ENTOMOLOGICAL OUTCOMES

Peiyu Liu[1], Libin Rong[1], Derek Cummings[2], Matt Hitchings[1]

[1]University of Florida, Gainesville, FL, United States, [2]Johns Hopkins University, Baltimore, MD, United States

Evaluating vector control interventions through randomized trials is often challenging because clinical endpoints, such as infection incidence, are rare and heterogeneous even areas at high risk, resulting in large required sample sizes. Antibodies to mosquito salivary proteins (MSPs) have emerged as promising markers of exposure to mosquito bites, yet their utility as trial endpoints is not fully understood. To address this gap, we developed a mechanistic modeling framework to describe MSP antibody dynamics in response to mosquito biting and to compare the statistical power of serological, clinical, and entomological trial endpoints. We introduce a new stochastic model for anti-MSP antibody dynamics---the Antibody Non-Homogeneous Poisson Process (ANPP) model---which incorporates seasonal variation in exposure and inter-individual heterogeneity. With a temperature-driven SEIR-SEI transmission model to create a unified simulation platform. Using this framework, we systematically compare sample size requirements for each endpoint under a range of simulated vector control strategies. Our results reveal a pronounced efficiency hierarchy: serological endpoints can reduce sample size needs by several orders of magnitude compared with clinical endpoints, especially in low-incidence settings. We also show that endpoints tied directly to mosquito population dynamics, such as antibody levels or trap counts, display seasonal patterns that mirror fluctuations in mosquito abundance, whereas infection-based endpoints remain comparatively flat. These findings provide a quantitative foundation for incorporating serological markers into trial design and highlight their potential to accelerate the evaluation and deployment of vector control tools.

7310

BLOOD STAGE MALARIA INDUCES TRAINED IMMUNITY IN HEPATOCYTES

Elizabeth KK Glennon[1], Tinotenda Tongogara[1], Nhi Ho[1], Primavera Veronica[1], Noah Stegman[1], Ling Wei[1], Lola Velush[1], Cassandra Fieldson[1], Bogdan Velychko[2], Ashley Subijanto[2], Sean C. Murphy[2], Raj P. Kapur[3], Nana K. Minkah[1], Nathan W. Schmidt[4], Tuan M. Tran[4], **Alexis Kaushansky**[1]

[1]Seattle Children's Research Institute, Seattle, WA, United States, [2]University of Washington, Seattle, WA, United States, [3]Seattle Children's Hospital, Seattle, WA, United States, [4]Indiana University School of Medicine, Indianapolis, IN, United States

Infectious diseases rarely exist in isolation. While they are often studied singly in the laboratory, pathogens in the field exist in the context of recurrent and co-occurring infections. *Plasmodium* parasites, the causative agents of malaria, are transmitted at high levels in endemic areas, with individuals experiencing hundreds of infectious bites a year. Adults in malaria endemic area develop partial immunity (control of parasitemia) and tolerance (reduced disease symptoms), with asymptomatic cases and prolonged low to submicroscopic parasitemia being a common feature. In this study, we use a sequential infection mouse model of chronic blood-stage *P. chabaudi,* with submicroscopic parasitemia, followed by *P. yoelii* liver stage infection, to assess the antigen-independent tuning of memory responses that shape the infection trajectory during *Plasmodium* sporozoite challenge in infection-experienced animals. We observed a reduction in *P. yoelii* liver-stage infection in *P. chabaudi* blood-stage-experienced animals that is independent of CD4 and CD8 T cell and antibody responses. Instead, we discovered evidence that blood-stage infection epigenetically reprograms the liver such that interferon-responsive genes, known to effectively regulate liver infection, are more accessible and are more rapidly transcribed in hepatocytes in response to a secondary stimulus. Moreover, we were able to recapitulate this phenotype when hepatocytes from *P. chabaudi* experienced animals were cultured *ex vivo* and stimulated with dsDNA. Our data are consistent with the hypothesis that hepatocytes can become epigenetically reprogramed in a manner akin to innate immune memory. To our knowledge, this is the first such report for hepatocytes in a mammalian system. It remains an open question to what extent *Plasmodium* training of hepatocytes might alter the trajectory of other infections or liver functions, and under what other conditions innate immune memory could be induced in hepatocytes.

7311

PFPIESP2 CONTRIBUTES TO BLOOD-BRAIN BARRIER DYSFUNCTION VIA EPHA2 WITH IMPLICATIONS FOR CEREBRAL MALARIA PATHOGENESIS

Alan A. Ardito, Philippa C. Vaughn-Beaucaire, Sunthorn Pond-Tor, Aidan S. Biondi, Sean E. Lawler, Jonathan D. Kurtis

Brown University, Providence, RI, United States

PfPIESP2, *Plasmodium falciparum* Parasite-Infected Erythrocyte Surface Protein 2, has been implicated in parasite adhesion to the human brain vasculature, and anti-PfPIESP2 antibodies have been correlated with protection from malaria in Malian children. Here, we tested whether recombinant PfPIESP2 disrupts *in vitro* models of the blood-brain barrier (BBB). In electrical cell-impedance sensing assays using HCMEC/D3 monolayers, PfPIESP2 induced a dose-dependent increase in endothelial permeability, which resolved upon protein removal. In 3D BBB spheroids composed of astrocytes, pericytes, and brain endothelial cells, treatment with PfPIESP2 increased spheroid permeability to macromolecules. To identify candidate host binding partners, we assessed PfPIESP2 binding to a panel of Eph receptors and found the strongest interaction with EphA2 by ELISA. Microscale thermophoresis confirmed PfPIESP2-EphA2 binding with high affinity (Kd = 13 nM). Consistent with receptor activation, treatment with PfPIESP2 induced EphA2 phosphorylation in HCMEC/D3 monolayers, which was abrogated by treatment with ALW-II-41-27, an EphA2 inhibitor. Because EphA2 can promote MLC2-mediated actomyosin contractility through Rho/ROCK signaling, we next examined phospho-MLC2 and found that PfPIESP2 increased MLC2 phosphorylation, an effect blocked by the ROCK inhibitor Y-27632. Together, these findings support a model in which PfPIESP2 engages human EphA2 to drive endothelial actomyosin contractility and BBB dysfunction during cerebral malaria pathogenesis. These results identify a candidate host-pathogen signaling pathway and suggest potential therapeutic targets in cerebral malaria.

7312

A PHYSIOLOGICALLY RELEVANT MOUSE MODEL OF MALARIA-ASSOCIATED ACUTE KIDNEY INJURY UTILIZING TRANSCUTANEOUS GFR MONITORING AND EXPANSION MICROSCOPY

Rafael B. Polidoro[1], Alleé Shaw[1], Fabricio Marcus Silva Oliveira[2], Sawyer Lehman[1], Evan Rajadhyaksha[1], Rose Odom[1], Andrea Conroy[1], Takashi Hato[1], Sabrina Absalon[1], Danielle Soranno[1], Nathan W. Schmidt[1]

[1]Indiana University School of Medicine, Indianapolis, IN, United States, [2]Washington, DC, United States

Malaria-associated acute kidney injury (MAKI) is a severe complication of *Plasmodium* infection, yet current animal models often rely on artificial surgical ischemia, genetic modifications, or specialized strains with antimalarials. To address this critical gap, we developed a physiologically relevant model utilizing simple C57BL/6 mice infected with *P yoelii*, relying on microbiota-enhanced disease severity rather than genetic modification or surgical intervention. This model demonstrates a distinct kinetic uncoupling of early inflammation and late-stage functional impact. By 10 days post-infection (dpi), prior to peak parasitemia, mice exhibit significant increases in systemic endothelial activation and anti-bacterial markers, alongside elevated urinary Kidney Injury Molecule-1 (KIM-1). However, overt functional kidney impairment measured in vivo via transcutaneous glomerular filtration rate (tGFR) monitoring and confirmed serum creatinine, does not peak until 18 dpi. Standard histopathological analysis reveals that glomerular and parenchymal immune infiltration significantly precedes this late-stage functional crash. To investigate the ultrastructural mechanisms driving this delayed injury, we applied Expansion Microscopy (ExM) to paraffin-embedded kidney sections. This high-resolution imaging revealed severe mesangial expansion, the distinct presence of parasitized red blood cells sequestered within the glomeruli causing impaired RBC flow, and immune activation. Furthermore, ExM uncovered a profound spatial shift in heme oxygenase-1 (HO-1) expression; while HO-1 is restricted to renin-producing cells at baseline, it is markedly upregulated in the proximal tubules and infiltrating immune cells post-infection. Together, these findings establish a robust, non-surgical model of MAKI, highlighting the delayed intrarenal impact and identifying structurally localized HO-1 and glomerular parasite sequestration as key features of malaria-induced renal failure.

7313

SYSTEMIC METABOLIC ALTERATIONS AND CACHEXIA BIOMARKERS IN NEWLY DIAGNOSED PULMONARY TUBERCULOSIS WITH SOIL TRANSMITTED HELMINTH COINFECTION

Prakash Babu Narasimhan, Komala Ezhumalai, Bindu Dasan, Arivoli Dhanasekaran, Jasemine Priyanga, Senbagavalli Prakash Babu, Sonali Sarkar

Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry, India

Tuberculosis (TB) is associated with weight loss, muscle wasting, and metabolic alterations, yet soil-transmitted helminth (STH) co-infection to cachexia-related pathways remains unclear. A total of 167 pulmonary TB patients from Puducherry and Tamil Nadu, India, were enrolled. *Ancylostoma duodenale*, *Necator americanus*, *Ascaris lumbricoides*, *Strongyloides stercoralis*, and *Trichuris trichiura* were analysed in stool by RT PCR. A panel of metabolic, gut-appetite and inflammatory (C-peptide, ghrelin, PYY, GLP-1, glucagon, APOA4, IL-6, leptin, amylin, secretin), and

muscle-wasting biomarkers (GDF-15, CNDP1, DKK3, MSTN, ACVA) in TB patients with and without STH co-infection was studied. Associations with BMI, smear grade, cavitation, inflammatory status, and muscle wasting measures (SNAQ, MUAC, handgrip strength) were analysed and then compared using t-tests, Mann–Whitney U, and χ^2 tests. STH co-infection was present in 37.12% (n=62, out of 167) of TB individuals. Clinical indicators showed greater disease severity in the STH co-infection group, with higher smear positivity (69% vs. 41%, p = 0.005) and high cavitation on chest X-ray (78% vs. 49%, p = 0.001). Lower handgrip and BMI in STH group compared to TB alone. Biomarkers for Appetite/ gut hormones like leptin, ghrelin, PYY, GLP-1, APOA4, glucagon and cachexia-associated markers like MSTN, CDNP1 and DKK3, were significantly elevated in both TB and TB-STH groups compared to healthy controls ($p < 0.05$, Dunn's test). To understand the inflammatory-metabolic–catabolic network, we did multivariable linear regression and Spearman correlations. IL-6 showed strong positive correlations with MSTN and DKK3, linking inflammation to muscle wasting. Helminth-specific modulation was observed in appetite hormones, suggesting subtle enhancement of anorectic signalling. Our data showed, TB-STH groups, MSTN, DKK3, and CDNP1 positively correlated with MUAC, grip strength, and BMI, more than the TB alone group. These findings underscore the need for integrated TB–STH management, particularly in endemic settings where co-infection is common and may worsen disease trajectory.

7314

LIVER FIBROSIS AND GENE EXPRESSION PATTERNS IN RABBITS INFECTED WITH TRICLABENDAZOLE-SUSCEPTIBLE AND -RESISTANT *FASCIOLA HEPATICA*: AI-BASED ANALYSIS

Timothy Wanninger[1], Vanessa Fernandez-Baca[2], Cristian Hoban[3], Choi Young-Jun[4], Claudia M. Trujillo-Vargas[1], Esteban Arroyave Sierra[1], Melinda B. Tanabe[5], Pedro Ortiz[3], Omar Saldarriaga[1], Heather Stevenson[1], Makedonka Mitreva[4], Miguel Mauricio Cabada[6]

[1]University of Texas Medical Branch, Galveston, TX, United States, [2]Universidad Peruana Cayetano Heredia, Cusco, Peru, [3]Universidad Nacional de Cajamarca, Cajamarca, Peru, [4]Washington University School of Medicine, St. Louis, MO, United States, [5]UTMB, La Marque, TX, United States, [6]University of Texas Medical Branch, Dickinson, TX, United States

Genotypic and phenotypic differences have been described between triclabendazole sensitive (TCBZ-S) and resistant (TCBZ-R) *Fasciola hepatica* parasites, but it is unclear if they produce different pathology. We used a rabbit model to study the contribution of fascioliasis to chronic liver disease in humans in endemic countries. Three groups of adult rabbits were challenged with 40 TCBZ-S metacercariae (n=5), 40 TCBZ-R metacercariae (n=4), or sham infection (n=3). Ninety days after challenge, the rabbits were euthanized, their livers were dissected, and were preserved in formalin. Twenty-four liver pathology slides (10 TCBZ-S, 8 TCBZ-R, and 6 controls) from the 12 rabbits were stained with Masson's trichrome and analyzed using Visiopharm software (v2021.09, Visiopharm A/S, Denmark) to quantify liver fibrosis (collagen-to-tissue ratio). We selected 4 liver biopsies per group to evaluate fibrosis-related gene expression using the nCounter® fibrosis panel (770 genes; Bruker Spatial Biology, Inc, Seattle, WA). The median number of adult flukes recovered was 9 (IQR 8-14) versus 9.5 (IQR 8.75-11) in livers infected with TCBZ-S or TCBZ-R flukes, respectively. AI-based image analysis demonstrated a mean overall collagen-to-tissue ratio of 0.085, with 0.12 in TCBZ-S and 0.09 in TCBZ-R groups. *F. hepatica* infection was associated with a higher collagen-to-tissue ratio compared to controls ($p < 0.001$), but no difference was observed between TCBZ-S and TBCZ-R groups (p=0.806), nor were there significant associations with egg counts (EPG; p=0.836) or parasite number (p=0.321). A total of 170 genes met detection and quality control criteria and were included in the differential expression analysis. ACTA2, COL14A1, and VIM genes were significantly upregulated in TCBZ-S versus controls, with no significant gene expression differences between TCBZ-R and controls. Ingenuity Pathway Analysis (IPA, Qiagen) revealed activation of extracellular matrix remodeling and wound healing pathways in TCBZ-S compared to controls, while most of them were absent in TCBZ-R. Our findings suggest that *F. hepatica* infection is associated with hepatic fibrosis.

7315

GENOMIC EPIDEMIOLOGY REVEALS ZOONOTIC AND PERSISTENT HUMAN TRANSMISSION OF *BRUGIA MALAYI* IN RESURGENT LYMPHATIC FILARIASIS

Young-Jun Choi[1], Irina Diekmann[1], Taniawati Supali[2], Rahmat Alfian[2], Yossi Destani[2], Noviani Sugianto[2], Mohd Hatta Abdul Mutalip[3], Nor Azlina Abdul Aziz[3], Khairiah Ibrahim[3], Kerstin Fischer[1], Peter U. Fischer[1], **Makedonka Mitreva**[1]

[1]Washington University School of Medicine, Saint Louis, MO, United States, [2]Universitas Indonesia, Jakarta, Indonesia, [3]Institute for Public Health, Setia Alam, Malaysia

The majority of human lymphatic filariasis (LF) cases in Indonesia are caused by *Brugia malayi*. Although Belitung District was declared LF-free in 2017 after five rounds of mass drug administration (MDA) and three transmission assessment surveys, adults with microfilaremia were detected in 2021. A similar resurgence was reported in Sabah District, Malaysia. To identify the sources of rebound infection and assess the role of animal reservoirs, we used genomic epidemiology to investigate transmission dynamics and host specificity by sequencing 348 individual *Brugia* spp. microfilariae from humans, cats, dogs, and monkeys across both countries. Population genomic analyses showed that parasites from Indonesia and Malaysia are genetically distinct from each other and from those in Thailand. In Indonesia, three genetically differentiated *B. malayi* populations were identified: two animal-associated lineages corresponding to Belitung and Mendanau Islands, and a third restricted to humans. Within each island, no genetic structuring was observed among non-human host species. The animal-associated lineages were also detected in humans on both islands, consistent with zoonotic spillover. However, persistent population structure in animal hosts across islands suggested limited spillback from humans to animals. Demographic analysis of the human-restricted lineage indicated a smaller effective population size and a bottleneck coinciding with earlier MDA, consistent with persistent human infections missed by surveillance. In Malaysia, a single *B. malayi* population infected humans and animals, supporting likely ongoing zoonotic transmission. Mitochondrial genome analysis identified a divergent haplogroup closely related to *B. timori* in both countries, revealing mitonuclear discordance and highlighting limitations of mitochondrial markers for species-level molecular surveillance. Together, these findings indicate that resurgent *B. malayi* infection is sustained by zoonotic reservoirs and persistent human transmission, underscoring the value of large-scale genomic surveillance for tailored LF control and elimination.

7316

EPIDEMIOLOGICAL INVESTIGATION OF LYMPHATIC FILARIASIS ANTIBODY-POSITIVE CASES DETECTED THROUGH POST-VALIDATION SURVEILLANCE IN NIUE, A LYMPHATIC FILARIASIS ELIMINATION SETTING

Adam T. Craig[1], Harriet L. S. Lawford[1], Grizelda Mokoia[2], Patricia Tatui[2], Andy Manu[2], Misiona Nicholas[2], Colleen L. Lau[1]

[1]The University of Queensland, Brisbane, Australia, [2]Niue Department of Health, Alofi, Niue

Lymphatic filariasis (LF) is a mosquito-borne neglected tropical disease targeted for elimination as a public health problem. Once eliminated, WHO strongly recommends post-validation surveillance (PVS) to detect resurgence and respond quickly. Niue is a small South Pacific island nation with a population of 1,600 people. Niue achieved WHO validation of LF elimination in 2016. In 2025, 9 years after validation and 21 years after the last mass drug administration (MDA), a near-census PVS survey was conducted. The survey included LF antigen (Ag) testing, microscopy of thick blood films for Ag-positive individuals, and antibody (Ab) testing using multiplex bead assays for all participants. Only one of 845 participants

tested Ag-positive, corresponding to a population Ag prevalence of 0.1% (95% CI: 0.02-0.88), indicating that elimination has been maintained. However, 10 individuals (six males, four females; age 11-68) were Ab-positive (five for *Wb 123*, four for *Bm 14*, and one dual-positive), including three born after cessation of MDA. A targeted investigation was undertaken to characterise potential exposure pathways. At least three individuals had recently migrated from LF-endemic countries, and one had spent an extended period in an endemic setting. Two had close family members who were Ag-positive in a 2009 LF survey. While there was no conclusive evidence of domestic transmission, the findings indicate possible recent exposure and warrant follow-up. In accordance with WHO guidance for post-elimination settings, periodic Ag and microfilaria testing alongside clinical monitoring of Ab-positive individuals is being considered. Although Ab testing is not yet routinely used for programmatic decision-making, these results highlight its potential as an early warning signal for ongoing transmission or resurgence. The findings raise important considerations for interpreting Ab results and highlight the potential of multiplex antibody-based surveillance approaches for LF detection in elimination settings. PVS remains essential to prevent resurgence in settings that have achieved elimination.

7317

TWENTY YEARS OF CARING FOR PATIENTS WITH LYMPHEDEMA INDUCED BY LYMPHATIC FILARIASIS IN PLATEAU AND NASARAWA STATES, NORTH CENTRAL NIGERIA: SUCCESSES, CHALLENGES AND PROSPECTS

Adamu Sallau[1], Bulus Mancha[1], Jenna E. Coalson[2], Emmanuel Emukah[1], Philemon Dagwa[3], Yakubu Adamu[4], Fatai Oyediran[5], Solomon Adelamo[1], Lindsay Rakers[2], Emily Griswold[2], Stephanie Palmer[2], Hannah Marie Lawinger[2], Abel Eigege[1], Emmanuel Miri[1], Gregory S. Noland[2]

[1]The Carter Center - Nigeria, Jos, Nigeria, [2]The Carter Center, Atlanta, GA, United States, [3]Plateau State Ministry of Health, Jos, Nigeria, [4]Nasarawa State Ministry of Health, Lafia, Nigeria, [5]Federal Ministry of Health, Abuja, Nigeria

Lymphatic filariasis (LF) transmission was interrupted in Plateau and Nasarawa states in 2012 through high coverage mass drug administration with ivermectin and albendazole. WHO LF elimination goals also require provision of care for patients suffering from chronic LF morbidities. In 2005, the Carter Center launched the formation of Hope Groups (HGs) for people with lymphedema (LE), aimed at providing community-based care and reducing disability and socio-economic burden. Health workers trained in LE management and mental health held patient care sessions twice yearly to offer the WHO recommended minimum care package. A total of 1,131 people (898 in Plateau; 233 in Nasarawa) have enrolled in HGs since inception. Simplified staging at enrollment showed 260 (23%) mild, 486 (43%) moderate, and 385 (34%) severe LE. Impact assessments comparing 2025 to 2021 baseline data among 591 patients showed significant improvements: the proportion of patients with entry lesions reduced from 230 (39%) to 53 (9%), fungal infections from 118 (20%) to 47 (8%), and acute attacks decreased from 153 (26%) to 65 (11%). Suitable footwear ownership improved from 212 (36%) to 419 (71%). However, some challenges remain. In 2025, 426 (72%) reported depression and feeling hopeless, 479 (81%) reported feeling self and public stigma, and 219 (37%) reported contemplating suicide. Overall, 85% of participants were satisfied and learned new skills to care for their LE. Challenges include weak health systems, misconceptions and myths about LF, low community awareness, irregular Hope Group attendance, long distance, and costs. To reduce the burden of mental health conditions and stigma, we recommend integrating HGs into the primary health care system, training more health workers on LE care and mental health, increasing community awareness and social support, and securing regular funding for disease prevention and disability inclusion from government sources for sustainability in places targeting LF elimination.

7318

HEALTH SYSTEMS BARRIERS TO POST-VALIDATION SURVEILLANCE FOR LYMPHATIC FILARIASIS: INSIGHTS FROM A NOMINAL GROUP TECHNIQUE AND EXPERT ELICITATION STUDY CONDUCTED IN THE PACIFIC ISLANDS

Adam T. Craig[1], Clement Couteaux[2], Ken Jetton[3], Roger Nehemia[4], Oliver Sokana[5], Tanebu Tong[6], Temea Bauro[6], Taulanga Baratio[6], Ofa Sanft Tukia[7], Joseph Takai[7], Satupaitea Viali[8], Noel Garma Soares[9], Maria Ome-Kaius[10], Mary Yohogu[11], Patricia Tatui[12], Litiana Volavola[13], Fasihah Taleo[14], Sala Saketa[15], Andie D. Tucker[16], Charles D. Mackenzie[16], Katherine Gass[16], Colleen L. Lau[1], Harriet L. S. Lawford[1]

[1]The University of Queensland, Brisbane, Australia, [2]Agence de Santé de Wallis et Futuna, Mata'Utu, Wallis and Futuna, [3]Republic of The Marshall Islands Ministry of Health and Human Services, Marjuro, Marshall Islands, [4]Te Marae Ora Cook Islands Ministry of Health, Rarotonga, Cook Islands, [5]Solomon Island Ministry of Health and Medical Services, Honiara, Solomon Islands, [6]Kiribati Ministry of Health and Medical Services, Tarawa, Kiribati, [7]Tonga Ministry of Health, Tongatapu, Tonga, [8]Oceania University of Medicine, Apia, Samoa, [9]Temor-Leste Ministry of Health, Dili, Timor-Leste, [10]PNG Institute of Medical Research, Madang, Papua New Guinea, [11]Papua New Guinea Ministry of Health, Port Moresby, Papua New Guinea, [12]Niue Department of Health, Alofi, Niue, [13]Fiji Ministry of Health and Medical Services, Suva, Fiji, [14]WHO, Port Vila, Vanuatu, [15]Pacific Community, Suva, Fiji, [16]Task Force for Global Health, Decatur, GA, United States

Lymphatic filariasis (LF) is a mosquito-borne neglected tropical disease that causes significant morbidity and social consequences. Efforts under the Global Programme to Eliminate Lymphatic Filariasis have substantially reduced prevalence, and 23 of 72 endemic countries have been validated as having eliminated the disease as a public health problem, including eight Pacific Island countries and territories. While post-validation surveillance (PVS) is strongly encouraged to ensure early detection of any resurgence, it has rarely been implemented, and barriers to its adoption remain poorly understood. We used two complementary qualitative approaches to identify systemic barriers and facilitators to LF-PVS in the Pacific Islands region. Firstly, we carried out a Nominal Group Technique, followed by a structured expert elicitation involving program managers and technical staff from Pacific islands that have achieved, or are nearing, elimination. Data were analyzed thematically and triangulated across sources. Seventy challenges were identified and organized into ten themes. Major barriers to LF-PVS implementation included poor understanding of LF-PVS options among health leaders, inconsistent technical and financial support, and a lack of operational guidance. Additional challenges involved the limited supply of rapid antigen tests used for field surveys, procurement delays, the absence of formal mandates, and community survey fatigue. Integrating PVS within existing health services was regarded by regional LF leaders as a way forward. These discussions have catalyzed a Pacific PVS Network to share knowledge, experience across Pacific and coordinate advocacy on PVS. As more countries reach LF elimination and transition to PVS, the findings and recommendations of this study will grow increasingly relevant.

7319

ASSESSING THE EFFECTIVENESS AND DISSEMINATION OF INFORMATION, EDUCATION, AND COMMUNICATION / BEHAVIOR CHANGE COMMUNICATION MATERIALS IN ONCHOCERCIASIS- AND LYMPHATIC FILARIASIS-ENDEMIC AREAS IN ETHIOPIA

Mengesha Abrha[1], Dazhanae Houston[2], Aderajew Mohammed[1], Yewondwossen Bitew[1], Geremew Haileyesus[1], Kassa Bulcha[1], Mustefa Abbamilki[1], Desalegn Jemberie[1], Tewodros Seid[1], Anley Haile[1], Zerihun Tadesse[1], Gregory S. Noland[3], Emily Griswold[3]

[1]The Carter Center - Ethiopia, Addis Ababa, Ethiopia, [2]Rollins School of Public Health, Emory University, Atlanta, GA, United States, [3]The Carter Center, Atlanta, GA, United States

Onchocerciasis (OV) and lymphatic filariasis (LF) impose major burdens in Ethiopia. High coverage mass drug administration (MDA) is essential to achieving elimination. Since 2001, programs have used educational outreach materials to raise awareness and encourage MDA uptake, yet little is known about their effectiveness or reach. This qualitative study examined use and perception of these materials in intervention areas. Purposive sampling across 12 districts in 5 regions included 34 focus group discussions (FGD), 57 in-depth interviews, 20 material dissemination interviews (IDI), and 29 flipchart usability tests conducted from May-August 2025. In total, 214 FGD participants (124 male) and 110 individuals (75 male), including community members, community drug distributors, and government health staff, provided feedback on MDA-related posters, flipcharts, and audio clips. Sessions were audiorecorded, transcribed, translated, and thematically analyzed. The posters included images of OV- and LF-associated symptoms. Participants said they had strong visual impact, were clearly understood, and motivated MDA uptake. Participants viewed them as trustworthy, culturally appropriate, and relevant, though some confusion remained between disease-specific prevention and general hygiene practices. Almost all FGD and IDI participants requested larger formats and locallanguage versions to improve accessibility. Dissemination gaps were notable; materials were mostly seen during MDA. Flipcharts had strong engagement when used interactively, but raised concerns about portability, facilitation skill, and inconsistent use. Audio clips were considered effective, but reach was limited, suggesting the need for dedicated community awareness days. While information, education, and communication materials improve knowledge and prevention, their impact is hindered by limited dissemination, small formats, and language constraints. Enhancing pretesting, prioritizing translations, increasing large-format and video-based materials, and ensuring consistent distribution will support OV and LF elimination.

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REDUCTION IN COMMUNITY PREVALENCE OF *ONCHOCERCA VOLVULUS* DNA IN *SIMULIUM* BLACK FLIES FOLLOWING LARGE COMMUNITY STUDY OF MOXIDECTIN PLUS ALBENDAZOLE IN CÔTE D'IVOIRE

Fidel Assamoa[1], Kerstin Fischer[2], Rishika Mallu[2], Peter Uwe Fischer[2], Benjamin Koudou[1], **Philip J. Budge**[2]

[1]Centre Suisse de Recherches Scientifiques en CI, Abidjan, Côte D'Ivoire, [2]Washington University School of Medicine, St. Louis, MO, United States

To assess the safety of moxidectin plus albendazole (MoxA) in a mass drug administration (MDA) setting, the MDGH-MOX-3002 study in Côte d'Ivoire treated over 5,000 individuals from May 2023 - Feb 2024 in the Akoupé health district. Study participants in three communities, Yadio, Assangbadji, and Ahéoua, were randomized in a 4:1 ratio to receive either MoxA or ivermectin plus albendazole (IA). In each of these areas, most community residents participated in the study. To document the effect of this near-community-wide treatment on transmission of *Onchocerca volvulus* in each community, we collected black flies via human landing catches from September - November each year, with 2419 black flies collected in 2023, 5902 in 2024, and 2970 in 2025. Only *Simulium damnosum s.l.* were analyzed; 2,360 (98%) of these were forest species. Biting rates were 14.75 flies/day/person. DNA was extracted from pools of 20 black fly heads using a standardized column methods and qPCR amplification using a Mic portable thermocycler and the O150 DNA repeat of *O. volvulus* as target. Preliminary analysis of tested pools showed that 24 of 78 (31%) pools from 2023 (baseline) were positive; 8/26 (31%) in Ahéoua, 14/38 (37%) in Assangbadji, and 2/14 (14%) in Yadio. Overall baseline estimated infectivity rate was 1.8% of flies (Poolscreen maximum likelihood prevalence [pMLP], 95% CI 1.2% - 2.6%). Among flies analyzed to date, 11/75 (17%) pools collected in in Assangbadji in 2024 are positive, for pMLP infectivity rate of **0.8%** (95% CI 0.4% - 1.4%), compared to **2.3%** (95% CI 1.3% - 3.7%) in the same area at baseline. These preliminary data suggest that near-community-wide treatment, in which 80% of participating individuals received MoxA, reduced *O. volvulus* prevalence in *Simulium* black flies by approximately two thirds over the course of one year. These data highlight the value of concurrent xenomonitoring in communities under treatment. A full analysis of collected but untested black flies from will provide more precise estimates of the effect in all study communities over time.

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HIGH SEROPREVALENCE OF ONCHOCERCIASIS AMONG PEOPLE WITH NODDING SYNDROME AND EPILEPSY IN MADI MID NORTH FOCUS, UGANDA

Elisa Byamukama[1], Jenna E. Coalson[2], Emalee Martin[2], David Oguttu[3], Edridah Tukahebwa Muheki[1], Edson Byamukama[1], Christine Nahabwe[3], Monica Kembabazi[3], Alfred Mubangizi[3], Lauri Bernard[2], Gregory S. Noland[2]

[1]The Carter Center - Uganda, Kampala, Uganda, [2]The Carter Center, Atlanta, GA, United States, [3]Ministry of Health, Kampala, Uganda

Madi-Mid North (MMN) focus in Uganda has undergone ivermectin mass drug administration (MDA) for onchocerciasis since 2012. Transmission is suspected to have been interrupted in the lower part of the focus, while treatments are ongoing in the upper part. MMN is also co-endemic for nodding syndrome (NS) - a progressive pediatric epilepsy of unknown etiology. Concerns about neurologic episodes may cause community drug distributors to withhold ivermectin administration from individuals with NS. This in turn may create reservoirs of parasite transmission if such individuals are systematically excluded from MDA. A cross-sectional epidemiological assessment was conducted in 2025 from Kitgum, Lamwo, Pader, Omoro and Gulu districts of MMN to determine the prevalence of *Onchocerca volvulus* (Ov) among people with a history of NS or epilepsy. Sub counties with the highest number of recorded NS cases were selected purposively. Targeted numbers per parish were determined proportional to the number of cases listed by health officers of each district. A total of 652 participants of all ages (median: 24 years) and sexes (42.5% female) were randomly selected from district case line listings. Dried blood spots (DBS) samples were collected and analyzed for Ov exposure using Ov16 enzyme-linked immunosorbent assay. Of these, 215 (33.0%, 95% confidence interval [CI] = 15.6 - 50.9%) were Ov16 antibody-positive, including 7 children aged 5-9 years old. 78 participants reported that they had never been treated with ivermectin, of which 22 (28.2%) were Ov16-positive. Skin-snips were collected from 210 Ov16-positive cases; only 1 was positive for Ov microfilariae by microscopy. Confirmatory testing with more sensitive molecular techniques is planned. These results reveal high Ov exposure among individuals with NS and epilepsy including among children 5-9 years old, suggesting ongoing local transmission. Targeted treatment strategies are needed to safely provide ivermectin treatment to this special population.

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SPATIO-TEMPORAL MODELLING OF ONCHOCERCIASIS PREVALENCE IN GHANA (1975-2015): DELINEATING TRANSMISSION HOTSPOTS TO SUPPORT TARGETED ELIMINATION

Millicent Opoku[1], David A. Dean[2], Kwadwo K. Frempong[3], Himal Shrestha[4], Dziedzom K. de Souza[3], Sellase Akosua Pi-Bansa[3], Joseph H. N. Osei[5], Joseph Opare[6], Ernest Mensah[6], Odame D. Asiedu[6], Warwick N. Grant[7], Daniel Boakye[8], Shannon N. Hedtke[1]

[1]La Trobe Institute of Molecular Sciences/Department of Microbiology, Anatomy, Physiology and Pharmacology, La Trobe University, Melbourne, Australia, [2]Department of Ecological, Plant and Animal Sciences, La Trobe University, Melbourne, Australia, [3]Department of Parasitology, Noguchi Memorial Institute for Medical Research, College of Health Sciences, University of Ghana, Accra, Ghana, [4]The Peter Doherty Institute, Melbourne, Australia, [5]Biomedical and Public Health Research Unit, Water and Research Institute, Council for Scientific and Industrial Research (CSIR), Accra, Ghana, [6]Neglected Tropical Diseases Programme, Disease Control and Prevention Department, Public Health Division, Ghana Health Service, Accra, Ghana, [7]Department of Microbiology, Anatomy, Physiology and Pharmacology, La Trobe University, Melbourne, Australia, [8]The END Fund/ Noguchi Memorial Institute for Medical Research, Legon, Ghana

Onchocerciasis (river blindness), a parasitic disease caused by the filarial nematode, *Onchocerca volvulus* and transmitted by *Simulium* blackflies, continues to pose a major public health burden in sub-Saharan Africa. Despite marked reductions in prevalence under the Onchocerciasis Control Programme and the African Programme for Onchocerciasis Control, through community-directed treatment with ivermectin (CDTI), achieving the World Health Organization elimination target, of sustained microfilarial (mf) prevalence below 1% depends on accurately identifying areas where transmission persists to enable targeted intervention strategies. Microfilarial prevalence data from 1,353 surveys across 671 villages were sourced from the Expanded Special Project for Elimination of Neglected Tropical Diseases and the Ghana Neglected Tropical Diseases Programme. Environmental and climatic covariates were organised into four conceptual transmission factors: climatic suitability, flowing water probability, vector blood-feeding likelihood, and bioregional land-use context. A Bayesian spatio-temporal zero-inflated beta-binomial model was implemented in Stan via brms in R. The model incorporated ecozone-specific temporal smooths, a two-dimensional spatial smooth and hierarchical random effects at ecozone and village levels. Prevalence exhibited pronounced spatial and temporal heterogeneity. Northern ecozones approached near-elimination levels by 2015, in contrast, the southern ecozone, particularly the semi-deciduous forest and forest-agriculture transition zones, declined more slowly. The Tano-Ankobra transmission zone was identified as a persistent transmission hotspot. Runoff probability and surface wetness showed positive associations with prevalence, reflecting *Simulium* dependence on fast-flowing water. This nationwide spatio-temporal assessment of onchocerciasis demonstrates persistent residual transmission in southern forested regions of Ghana and underscores the need for intensified targeted interventions to achieve elimination.

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COMMUNITY KNOWLEDGE, ATTITUDES, AND PRACTICES ON ONCHOCERCIASIS AND MASS DRUG ADMINISTRATION IN METEKEL ZONE, NORTHWEST ETHIOPIA

Alemayehu Assefa[1], Aderajew Mohammed[1], Mengesha Abrha[1], Desalegn Jemberie[1], Nibretu Tarekegn[1], Anley Haile[1], Tewodros Seid[1], Yewondwossen Bitew[1], Mustefa Abbamilki[1], Meberatu Beguno[2], Kibru Sibani[2], Emily Griswold[3], Jenna E. Coalson[3], Gregory S. Noland[3], Zerihun Tadesse[1]

[1]The Carter Center - Ethiopia, Addis Ababa, Ethiopia, [2]Benishangul Gumuz Regional Health Bureau, Assosa, Ethiopia, [3]The Carter Center, Atlanta, GA, United States

Onchocerciasis persists in Metekel zone, Benishangul-Gumuz Region, Ethiopia despite longterm community-directed treatment with ivermectin (CDTI). Supervision reports from mass drug administration (MDA) campaigns indicate persistent gaps in community knowledge and practices. A knowledge, attitudes, and practices (KAP) study was undertaken to identify barriers to MDA compliance and generate insights to tailor ongoing elimination interventions. The community-based cross-sectional survey was conducted in January 2025, across three districts and Gilgel Beles town. Adults (≥18 years) were selected in house-to-house interviews using multi-stage stratified sampling. Data were collected electronically on ODK Collect. Custom KAP indicators were scored, and analyses were performed using SPSS v25. Among 842 respondents (66% female; mean age 37.2 years), 61% were aware of onchocerciasis, but only 9% demonstrated high knowledge. Misconceptions were common, with just 33% identifying black fly bites as the mode of transmission. Most (62%) perceived onchocerciasis as low risk, especially younger adults, and the majority (82%) perceived the benefits of MDA as high. Males reported more barriers to MDA than females. MDA compliance was 75% overall, higher among females and younger age groups. About 24% reported supporting the CDTI program beyond medication intake, including transporting MDA materials, mobilizing communities, and busting myths, with higher participation among those aged 56-65. Health workers (57%) and community drug distributors (38%) were the main sources of onchocerciasis information. Moderate awareness, but low specific knowledge and low perceived risk of onchocerciasis, may be barriers to achieving elimination targets in Metekel zone. Tailored health education targeting males, middle-aged groups, and poorly informed occupational categories is needed. Strengthening community engagement, improving community drug distributor training, and refining health education materials can enhance compliance and accelerate progress toward elimination.

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RESULTS FROM A PHASE 2 SINGLE-BLIND, RANDOMIZED, CONTROLLED STUDY TO EVALUATE THE SAFETY AND IMMUNOGENICITY OF A 2-DOSE SCHEDULE OF THE ALTSONFLEX1-2-3 GMMA VACCINE AGAINST S. SONNEI AND S. FLEXNERI 1B, 2A, 3A IN AFRICAN INFANTS

Josphat Kosgei[1], Maxime Zidan[2], Mohamed Oujaa[2], Francesca Mancini[2], Giulia Luna Cilio[3], Eray Salt[4], Pietro Ferruzzi[2], Simona Nigro[2], Omar Rossi[2], Valentino Conti[2], Nuria Martinez-Alier[2], Francesca Micoli[2], **Ashwani Kumar Arora**[2]

[1]Kenya Medical Research Institute/U.S. Army Medical Research Directorate-Africa/Kenya-Henry Jackson Foundation MRI, Kericho, Kenya, [2]GSK Vaccines Institute for Global Health, Siena, Italy, [3]GSK, Siena, Italy, [4]GSK, Warsaw, Poland

Shigellosis is a major cause of pediatric diarrheal disease and mortality in low- and middle-income countries (LMICs), with no licensed vaccines for global use and rising antibiotic resistance. We report the safety and immunogenicity of altSonflex1-2-3, a 4-component GMMA-based vaccine presenting serotype-specific lipopolysaccharide (LPS)/O-antigen (OAg) targeting *Shigella (S) sonnei* and *S. flexneri* serotypes 1b, 2a, and 3a. This Phase 2 study (NCT06663436; EudraCT 2023-000945-18) evaluated a 2-dose schedule in infants. Healthy 9-month-old infants (N=200) in Kenya were randomized (1:1:1:1) to receive two intramuscular doses (Day (D)1 and D 169) of altSonflex1-2-3 at three dose levels—Dose A (3.75 µg), Dose B (7.5 µg), or Dose C (15 µg) of OAg per component—or control vaccines (D 1: TYPHIBEV typhoid vaccine; D 169: Infanrix hexa). A measles-rubella vaccine (MR-VAC) was co-administered to all groups on D 1 and D 169. Safety and immunogenicity were assessed. No safety concerns were identified. Solicited events tended to be more frequent in altSonflex1-2-3 groups than in the control group; pain was the most frequent solicited event. Unsolicited adverse events tended to be less frequent in altSonflex1-2-3 recipients than in controls. The vaccine was similarly immunogenic across dose levels. By ELISA, anti-*Shigella* LPS/OAg IgG GMCs increased at 28 days post-each dose vs baseline in altSonflex1-2-3 groups: *S. sonnei* ~6-8 fold post dose 1 and ~107-160 fold post dose 2; *S. flexneri* 2a ~14-18 fold post dose 1 and maintained post dose 2; *S. flexneri* 1b and 3a ~3-5 fold post dose 1 and ~4-6-fold post dose 2. Strong serum bactericidal activity was induced with fold rises (Dose A vs baseline) at 28

days post dose 1/2 of: *S. sonnei* 10.5/55.8; *S. flexneri* 1b 4.9/10.9; 2a 34.9/75.2; 3a 3.3/6.2. No clear immune interference on MR-VAC responses was observed with co-administration. The 2-dose altSonflex1-2-3 schedule induced strong, functional, serotype-specific immune responses in infants, with no safety concerns identified that would preclude further development.

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ASSESSMENT OF ANTIBODY TITER TRAJECTORIES IN A CONTROLLED HUMAN INFECTION MODEL CHALLENGE WITH *SHIGELLA FLEXNERI 2A* AND *S. SONNEI*

Madeleine Blunt[1], Subhra Chakraborty[2], Jessica Brubaker[1], Lou Bourgeois[1], Chad Porter[3], Katherine DeTizio[4], Sandra Isidean[5], Kawsar R. Talaat[6]

[1]Johns Hopkins, Baltimore, MD, United States, [2]Johns Hopkins University, Baltimore, MD, United States, [3]Naval Medical Research Ctr, Silver Spring, MD, United States, [4]Naval Medical Research Command, Silver Spring, MD, United States, [5]Silver Spring, MD, United States, [6]Johns Hopkins Univ, Baltimore, MD, United States

Shigella sp. causes substantial disease burden, predominately in low-middle income country pediatric populations, and also effects travelers and deployed military personnel. Vaccine development has been prioritized, but is complicated by the fact that immunity to *Shigella* appears to be species-specific (based on the O antigen) and there is no consensus for an immunological correlate of protection. More conserved proteins may be useful in vaccine development. *Shigella* uses conserved invasion proteins (IpaA-D) to penetrate the mucosal layers of the terminal ileum, colon, and rectum. These effector proteins are shared across all *Shigella* species, making them a potential candidate for broadly protective vaccine. We conducted a longitudinal data analysis of *Shigella* species-specific LPS, IpaB and IpaC serum antibodies in individuals who had undergone challenge with either *S. flexneri* 2a (2457T) or *S. sonnei* (53G) at the JHU CIR in 2023. Our goal was to determine differences in response profile trajectories by serotype or among protected and unprotected participants. Forty-six naive volunteers were initially challenged with one serotype and, after 4 months, re-challenged with the other serotype to evaluate cross-species protection. Our analyses were limited to the 29 participants who participated in the rechallenge. We used Welch T-tests and a linear mixed model to examine the association between antibody titer mean/trajectory and *Shigella* serotype. For IpaB and IpaC there were no statistical differences in mean max titer for IgG or IgA nor in the trajectory of IgG antibody titers between *Shigella* serotypes after both challenge events. However, the trajectories for LPS IgG and IgA titers were significantly different between participants challenged with *S. flexneri* and *S. sonnei* after rechallenge, corresponding to differences in symptoms upon rechallenge. This longitudinal analysis revealed the potential ability to distinguish susceptibility to *Shigella* based on a temporal LPS IgA antibody titer trajectory.

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ASSESSING THE IMPACT OF THE WASHMOBILE PROGRAM IN THE DEMOCRATIC REPUBLIC OF THE CONGO ON TYPHOIDAL *SALMONELLA* SEROINCIDENCE: A RANDOMIZED CONTROLLED TRIAL

Christine Marie George[1], Presence Sanvura[2], Jean-Claude Bisimwa[2], Kelly Endres[3], Alves Namunesha[2], Willy Felicien[2], Blessing M. Banywesize[2], Camille Williams[3], David Allen Sack[3], Raissa Boroto[2], Gisèle Nsimire[2], Feza Rugusha[2], Freddy Endeleya[2], Pacifique Kitumaini[2], Claude Lunyelunye[2], Emmanuel Buhendwa[2], Pascal K. Bujiriri[2], Jessy Tumusifu[2], Brigitte Munyerenkana[2], Shubhanshi Trivedi[4], Kilee Davis[5], Kenadee Jacbonson[5], Khaled M. Al-Dandashi[5], Laura Caulfield[6], Justin Bengehya[7], Ghislain Maheshe[2], Cirhuza Cikomola[2], Alain Mwishingo[8], Richelle Charles[9], Lucien Bisimwa[2]

[1]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [2]Université Catholique de Bukavu, Bukavu, Democratic Republic of the Congo, [3]Baltimore, MD, United States, [4]University of Utah - Division of Infectious Diseases, Lehi, UT, United States, [5]University of Utah, Salt Lake City, UT, United States, [6]John Hopkins Univ, United States, [7]Bureau de l'Information Sanitaire, Surveillance Epidémiologique et Recherche Scientifique, Division Provinciale de la Santé Sud Kivu, Ministère de la Santé, Bukavu, Democratic Republic of the Congo, [8]Bukavu, United States, [9]Massachusetts General Hospital, Boston, MA, United States

Targeted WASH can be a cost-effective approach during outbreaks to reduce the spread of infectious diseases by targeting the highest risk populations. In partnership with the Ministries of Health in the Democratic Republic of the Congo (DRC) and Bangladesh, we developed the WASHmobile program for diarrhea patient households and those in diarrhea outbreak areas. In this study we assessed the impact of WASHmobile on *Salmonella enterica serovar* Typhi (*S.* Typhi) serologic responses in the DRC. The WASHmobile RCT enrolled diarrhea patient households in Bukavu, DRC. Households were randomized into two arms: single in-person visit for the DRC government's standard message on oral rehydration solution use and a basic WASH message (standard arm); or this standard message and the WASHmobile program with weekly voice and text messages and quarterly in-person visits (WASHmobile arm). Serum samples were analyzed for IgG and IgA responses to HlyE, an antigen-specific to typhoidal *Salmonella*. A *S.* Typhi infection was defined by a greater than or equal to fourfold rise in HlyE IgA or IgG antibody levels during at least one of the following time intervals during the 12-month surveillance period: baseline to 1-month timepoint, baseline to 6-month timepoint, and 6-month to 12-month timepoint. One hundred ninety-six participants between 6-59 months of age were enrolled in this study: 108 WASHmobile arm and 88 standard arm. *S.* Typhi infections were significantly lower in the WASHmobile arm compared to the standard arm during the 12-month study period (6% vs. 14%) (odds ratio: 0.36 [95% confidence interval: 0.13, 0.99]), with adjustment for age, gender, and diarrhea patient status. The WASHmobile program significantly reduced *S.* Typhi infections during the 12-month program period among children under 5 years of age in the DRC. This is consistent with RCT findings that WASHmobile reduced *Vibrio cholerae* serological markers and diarrhea healthcare facility visits. These findings suggest that the WASHmobile program can be used as an potential approach to reduce *S.* Typhi infections among young children in the DRC.

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COMPREHENSIVE CHARACTERIZATION OF THE HUMORAL RESPONSE ELICITED BY A TRIVALENT VACCINE AGAINST INVASIVE NONTYPHOIDAL SALMONELLOSIS AND TYPHOID FEVER IN AFRICAN ADULTS

Francesca Mancini, Beatrice Grossi, Marta Benincasa, Daniele De Simone, Chiara Fineschi, Federica Boretto, Yasir Shitu Isa, Rita La Gaetana, Elisa Marchetti, Nuria Martinez-Alier, Simona Rondini, Conti Valentino, Ashwani Kumar Arora, Rocio Canals Alvarez, Omar Rossi

GSK Vaccines Institute for Global Health (GVGH), Siena, Italy

Invasive nontyphoidal salmonellosis (iNTS), primarily attributable to *Salmonella* Typhimurium and *S.* Enteritidis, and typhoid fever remain major public health burdens in sub-Saharan Africa. With no licensed iNTS vaccine and limited implementation of typhoid conjugate vaccines (TCVs), we developed a trivalent iNTS-TCV vaccine candidate combining iNTS Generalized Modules for Membrane Antigens (iNTS-GMMA) and TCV. Its safety and immunogenicity were assessed in a phase 1/2a study in Belgium and Malawi (NCT05480800). In Malawi, 105 healthy adults were randomized (3:3:1) to receive, in different arms, iNTS-TCV and saline, or iNTS-GMMA and TCV, or a comparator vaccine and saline, on Days 1, 57, and 169. Serological analysis on sera from Malawian adults showed that Vi-polysaccharide-specific and O-antigen (OAg)-specific IgG responses increased post-first dose with no boosting after subsequent doses, and immunogenicity profiles were comparable across iNTS vaccine regimens. Serum bactericidal activity (SBA) rose against *S.* Typhimurium. This work expands the humoral response analysis to include IgG subclasses, IgA and IgM, and SBA against additional *Salmonella* strains. Comparably to iNTS-GMMA+TCV, iNTS-TCV elicited robust Vi-specific IgA responses and low

S. Typhimurium and *S.* Enteritidis OAg-specific IgA responses. A modest OAg-specific IgM response was observed upon vaccination with iNTS-TCV only among participants with non-detectable baseline antibodies and only for *S.* Enteritidis. Vi-specific IgG1 and IgG2 were detected in both iNTS-TCV and iNTS-GMMA+TCV groups. SBA against *S.* Dublin, *S.* Typhimurium 4/74, and *S.* Typhimurium 10433_3 was detected after two doses of iNTS-TCV. *S*. Typhimurium OAg-specific IgM correlated with bactericidal activity, while *S.* Enteritidis OAg-specific IgM did not. Overall, iNTS-TCV and iNTS-GMMA+TCV induced robust and comparable humoral responses across tested Ig isotypes and functional antibodies against strains carrying OAg features not represented in the vaccine. Further evaluation will be done in the infant target population to confirm the kind of immune response elicited by the iNTS-TCV.

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USE OF AMPLIFICATION-BASED DIAGNOSTIC TEST (RLDT) FOR *SHIGELLA* DIAGNOSIS IN BANGLADESH

Farina Naz[1], Sampa Dash[1], Eva Sultana[1], Mohammad Ali[1], Shaniram Das[1], Md. Razibur Rahman[1], Mizan Khan[1], ASG Faruque[1], Subhra Chakraborty[2]

[1]icddr,b, Dhaka, Bangladesh, [2]Johns Hopkins University, Baltimore, MD, United States

The current lack of diagnostic capabilities in many parts of the world to detect and treat shigellosis among children has been a major barrier to controlling this disease. Clinical manifestations are often non-specific, leading to misdiagnosis or delayed treatment and increased antibiotic resistance. The majority of laboratories do not have simple, rapid, sensitive tests available to diagnose shigellosis. The Rapid Loop-mediated Isothermal Amplification-based Diagnostic Test (RLDT) offers a simple, cost-effective, and practical solution, combining robust molecular performance with ease of use in real-world settings. Evidence from a series of studies in Bangladesh suggests that RLDT is well suited for broader use. Validation of RLDT in Bangladesh demonstrated that the method had high agreement with fresh stool and performed consistently well with rectal swabs and alternative transport media, with good sensitivity (>97.6%) and very high specificity. In a rural primary healthcare facility, laboratory staff were able to perform RLDT independently after brief training, achieving 98% sensitivity and 99% specificity, with results closely matching those from gold standard laboratory staff (κ=0.96), and the test was well accepted regardless of routine resource constraints. Ongoing INSIGHT study findings further demonstrate the value of implementing RLDT assay in rural settings; among 5,815 stool samples from diarrheal children seeking care at the hospitals tested with RLDT for *Shigella*, 1,823 were positive for *Shigella* (31.35%). An additional 1,656 children were tested at the community, bringing the total to 7,909 across Mirzapur, Tangail, and Dhaka in Bangladesh. Notably, RLDT identified more *Shigella*-positive cases (> 2-fold) than culture, the conventional gold standard, indicating improved sensitivity and detection of infections that might otherwise be missed. Taken together, these findings show that RLDT is a practical and scalable diagnostic assay, supporting its wider integration into routine diagnostics and strengthening surveillance and public health systems in *Shigella*-endemic settings.

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TREATMENT OUTCOMES OF AZITHROMYCIN IN DRUG RESISTANT SHIGELLOSIS IN BANGLADESH.

Subhra Chakraborty[1], Fan He[1], Sampa Dash[2], Eva Sultana[1], Jamie Perin[3], Camille Williams[3], Farina Naz[1], Mohammad Ali[1], Abu S.G. Faruque[4]

[1]Johns Hopkins University, Baltimore, MD, United States, [2]International Centre for Diarrheal Disease Research, Bangladesh (icddr,b), Dhaka, Bangladesh, [3]Baltimore, MD, United States, [4]icddr,b, Dhaka, United States

Shigella is a primary cause of moderate-to-severe diarrhea in children living in impoverished areas of the world. It is a prime target for vaccine development, although no approved vaccine is presently available. Azithromycin is frequently used to treat shigellosis; however, resistance to azithromycin and other antibiotics is increasing, and limited data are available regarding the clinical outcomes of azithromycin treatment of shigellosis. We performed an observational study in Bangladesh of children under 5 years old seeking care for diarrhea in the study hospitals and were *Shigella* positive. The children were treated with Azithromycin. Susceptibility testing was performed by disk diffusion, and minimum inhibitory concentrations (MIC) were determined post hoc on all *Shigella* isolates, and clinical response was correlated with in vitro susceptibility. A total of 135 *Shigella* culture-positive patients were included in the primary analysis. *Shigella flexneri* (56.3%) was more common than *S. sonnei* (43.7%). Only two isolates were *S. boydii.* Infection with *Shigella* with decreased susceptibility to azithromycin was significantly associated with a longer persistence of diarrhea (median 3.88 vs. 2.65 days; 95%CI 0.04 -1.71; p<0.05), higher persistence of culture positivity for more than 4 days (67.5% vs. 10%) and longer duration of hospitalization (median 20 vs. 11.5 hours; 95% CI 0.5 - 15; p ≤0.05). *S. sonnei* constituted most of the isolates with decreased susceptibility to azithromycin (86.4%) and thus, poor clinical outcomes were associated with *S. sonnei,* while only 23.7% of the *S. flexneri* isolates had decreased susceptibility to azithromycin. The MIC of azithromycin was >256µg/mL for the majority of the *S. sonnei* isolates (88.6%) with reduced susceptibility. This study documents significantly worse clinical outcomes for shigellosis with decreased susceptibility to azithromycin and showed that *S. sonnei* is a drug-resistant threat.

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DEFINING A SEROLOGIC SIGNATURE THAT DISTINGUISHES *S.* TYPHI FROM *S.* PARATYPHI A INFECTION

Hiroki Yamaguchi[1], Claire Evelyn Munroe[1], Edward T. Ryan[1], Md Taufiqur Rahman Bhuiyan[2], Farhana Khanam[2], Firdausi Qadri[2], **Richelle Charles**[1]

[1]Massachusetts General Hospital, Boston, MA, United States, [2]International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Dhaka, Bangladesh

Enteric fever, mainly caused by *S.* Typhi and *S.* Paratyphi A, remains a major public health concern. While *S.* Typhi is responsible for most cases, *S.* Paratyphi A causes 10-50% and is not covered by existing vaccines. Clinically, typhoid and paratyphoid fevers are indistinguishable, yet accurate serovar identification has important implications for vaccine policy, clinical management, and surveillance, especially given the markedly greater antimicrobial resistance (AMR) observed in *S.* Typhi compared to *S.* Paratyphi A. Conventional blood culture-based, if available, is slow while commercially available rapid serodiagnostics lack specificity and sensitivity. Although, there are promising rapid diagnostic tests in the pipeline based on detecting IgA responses to HlyE and *S*. Typhi LPS, this assay is unable to discriminate between the two serovars. To address this gap, we evaluated antibody responses to *S*. Typhi and *S*. Paratyphi A lipopolysaccharide (LPS) antigens in culture-confirmed typhoid (n=108) and paratyphoid cases (n=36) from Bangladesh. Although cross-reactivity to LPS was noted, responses were strongest to the homologous serovar. The O9/O2 IgM ratio (Typhi LPS/Paratyphi LPS) demonstrated the strongest discriminatory performance, with high AUCs in children <5 years (0.95) and 5-15 years (0.99), and slightly lower but still good performance in adults >15 years (0.80). IgA showed moderate discrimination, with AUCs ranging from 0.53 to 0.69, performing best in children <5 years. IgG had the weakest and most variable performance, with AUCs between 0.52 and 0.73, showing its highest discrimination in the 5-15 year age group. Overall, IgM consistently outperformed IgA and IgG across all ages, particularly in pediatric populations in discriminating *S*. Typhi from *S*. Paratyphi infections. A diagnostic tool capable of distinguishing between these serovars has potential for improving clinical management of enteric fever, monitoring vaccine impact, identifying epidemiologic shifts, and guiding next-generation vaccine development efforts that will include bivalent vaccines protecting against both typhoid and paratyphoid fever.

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ASSESSING THE DIAGNOSTIC PERFORMANCE OF ANTI-HEMOLYSIN E AND LIPOPOLYSACCHARIDE ANTIBODIES FOR TYPHOID INTESTINAL PERFORATION IN TYPHOID-ENDEMIC SETTINGS IN NIGER

Ezekiel Bonwin Ackah[1], Beatrice Nyantakyiwaa[1], Isaac Kissi[1], Katherine Shafer[2], Leah Sukri[3], Laura Hobbs[3], Michael Owusu[1], Issoufou Samaila[2], Shrimati Datta[3], Claire Munroe[4], Yaw Adu-Sarkodie[1], Ellis Owusu-Dabo[1], Jason R. Andrews[5], Kathleen Neuzil[3], Yakoubou Sanoussi[2], Matthew B. Laurens[3], Leslie Jamka[3], Kristen Aiemjoy[6], Richelle Charles[4]

[1]Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, [2]Hopital de la SIM Galmi, Biel, Niger, [3]Center for Vaccine Development and Global Health (CVD), University of Maryland School of Medicine, Baltimore, MD, United States, [4]Massachusetts General Hospital, Boston, MA, United States, [5]Stanford University, Stanford, CA, United States, [6]University of California Davis, Fair Oaks, CA, United States

Typhoid intestinal perforation (TIP) is a fatal late complication of *Salmonella* Typhi infection, often negative on blood culture. The IgA and IgG antibodies (Abs) against *S*. Typhi hemolysin E (HlyE) and lipopolysaccharide (LPS) antigens (Ags) have emerged as markers for typhoid diagnosis. Yet, their potential to diagnose TIP is not known. We evaluated the potential of these Abs to diagnose TIP among patients undergoing emergency laparotomy for atraumatic intestinal perforation (IP) in Niger. Dried blood spots (DBS) were collected pre- and 1-month post-surgery (pre- and post-op), and IPs typed as TIP or non-TIP based on surgical analysis. The Abs were measured using kinetic enzyme assays: Plates were coated overnight and blocked; samples containing the Abs were added; enzyme-linked anti-human Abs and substrates were then added; enzyme activities were measured at 450nm, from which Ab levels were derived and reported in ELISA units. The diagnostic potential was assessed using ROC and AUC analyses in R. Optimal cutoffs were found using Youden's index to maximize sensitivity and specificity. Overall, DBS was collected from 424 patients aged 3-17 years with TIP (n=333) or non-TIP (n=91); IP cause of non-TIPs were confirmed (control, n=48) or unconfirmed (n=43; excluded from analysis). Each Ag-Ab isotype discriminated between TIP and controls, AUC, 95% CI: HlyE IgA (0.91, 0.0.86-0.0.96); HlyE IgG (0.94, 0.90-0.99); LPS IgA (0.92, 0.86-0.98); LPS IgG (0.96, 0.92-1.00). Compared to each Ag-isotype or their combination, LPS IgG showed the best performance with 98% sensitivity and 92% specificity. The LPS IgG AUC (95% CI) values increased to 0.97 (0.92-1.00) among the ≥10 years with 98% sensitivity and 97% specificity. Compared with pre-op, the post-op AUC values were similar or lower for each Ag-Ab isotype or in combination. The results show that these Abs have the potential to diagnose TIP in resource-poor settings. Integration of anti-HlyE and LPS serology into surveillance workflows could improve typhoid detection and burden estimation. Further studies are needed to validate such potential in children <10 years.

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WHOLE GENOME SEQUENCING OF ENTERIC ISOLATES IDENTIFIES ANTIMICROBIAL RESISTANCE GENES AND VIRULENCE FACTORS IN *ESCHERICHIA COLI* AND *SHIGELLA* ISOLATES

Chanel Mosby-Tourtellot[1], Lidimarie Trujillo Rodriguez[1], Adrian Paskey[1], Andrea Luquette[1], Anthony Cicalo[1], Maren Fitzpatrick[1], Ghyssella Valdiviezo[1], Kyle Long[1], Hunter Smith[2], Mathew Esona[3], Regina Zing Cer[1], Francisco Malagon[1], Cameron Sayer[1], Francisco Mimica Porras[3], Kimberly Bishop-Lilly[1]

[1]Naval Medical Research Command, Fort Detrick, MD, United States, [2]Armed Forces Health Surveillance Division, Silver Spring, MD, United States, [3]Naval Health Research Center, San Diego, CA, United States

Bacterial acute gastroenteritis (AGE) is a major public health concern that has become increasingly complicated to treat due to the rise of antimicrobial resistance (AMR). The sudden onset of diarrhea that can accompany AGE poses a threat to the operational readiness of military personnel due to recovery timelines and potential complications in treatment due to AMR. To gain insight into the presence of AMR genes and virulence factor (VF) genes in bacterial species infecting the military and civilian population of the southwest U.S.A, 79 isolates consisting of 38 *Escherichia coli* and 41 *Shigella* spp. were collected from patients diagnosed with AGE. These isolates were sequenced using both long- and short-read platforms, resulting in high-quality assemblies of >99% completeness. These assemblies were analyzed for genomic characterization, including core genome phylogeny and identification of plasmids, AMR genes, and VF genes. The 38 *E. coli* isolates represented enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), *Shiga* toxin-producing *E. coli* (STEC), enteroinvasive *E. coli* (EIEC), and enteroaggregative *E. coli* (EAEC) pathotypes. Our analyses identified an average of 53.5 AMR genes per isolate, most of which were located on the chromosome and included the presence of class I and II integrons that duplicate AMR genes within isolates. Multidrug resistance efflux pumps were the most prevalent resistance mechanism across all isolates. The AMR genes found confer resistance to over 14 drug classes, including beta-lactams, fluoroquinolones, and aminoglycosides. Similarly, VF genes were found in all isolates, with an average of 20.3 VF genes per isolate, most of which were also detected on the chromosome. Presence of the AMR/VF genes and BLAST results were used to classify *Shigella* spp. as either *S. sonnei* or *S. flexneri*. In this work, we performed advanced genetic characterization, identified AMR and VF genes, and provided additional details associated with each pathotype. This work helps support mission readiness by informing treatment options for the Military Health System.

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MESOPHILIC ANAEROBIC DIGESTION AS A POTENTIAL STRATEGY FOR THE CONTROL OF *TAENIA SOLIUM* AND OTHER TAENIID TRANSMISSION IN ENDEMIC SETTINGS

Brenda L. Gonzales[1], Yeny Mamani-Mendoza[1], Nicol Avila-Flores[2], Miriam T. Colachagua[1], Ana C. Ho-Palma[3], Maria T. Lopez-Urbina[1], Daniel A. Andrade[4], Hector Hugo Garcia[4], Sarah Gabriël[5], Javier A. Bustos[4], Luis A. Gomez-Puerta[1], Cesar M. Gavidia[1], Seth O'Neal[6], Eloy Gonzales-Gustavson[1]

[1]Universidad Nacional Mayor de San Marcos, Lima, Peru, [2]Asociación Benéfica Prisma, Lima, Peru, [3]Universidad Nacional del Centro del Perú, Huancayo, Peru, [4]Universidad Peruana Cayetano Heredia, Lima, Peru, [5]Universiteit Gent, Ghent, Belgium, [6]Oregon Health & Sciences University, Portland, OR, United States

Mesophilic anaerobic digestion (MAD) is a technology used for the treatment of waste and the production of renewable energy, mainly in low- and middle-income countries. It uses biodigesters to generate biogas and a nutrient-rich effluent that can be used as fertilizer, making it an attractive option for rural communities with limited sanitation and energy infrastructure. Although previous studies have reported adequate biosafety of MAD effluents, these have primarily focused on bacterial pathogens, while highly pathogenic cestodes such as *Taenia solium*, capable of causing severe central nervous system infections and environmental contamination through effluent, have not been studied. Thus, it is necessary to determine whether MAD destroys cestodes eggs or whether they retain viability and potential infectivity after exposure to the system. In this context, this study is being conducted in the Central Highlands of Peru using a 1 m³ MAD system. Nylon bags (10 µm pore) containing eggs of *T. solium* (2,000), *E. granulosus* (5,000), or *T. hydatigena* (20,000) were prepared, each species in separate bags, and introduced into MAD and environmental matrices (water and soil). At defined time points (days 0, 7, 14, 30, 60, 90, and 180), six bags from MAD and six from environmental matrices (three water, three soil) are retrieved to assess total eggs recovered, egg hatching into oncospheres, oncosphere viability by trypan blue staining, and activation with porcine bile. On day 7, a greater than 95% reduction in the number of eggs recovered under MAD conditions is observed for all three parasites, while most of the remaining eggs for each parasite maintain viability in vitro; in contrast, no reduction is observed in the eggs of any parasite in water and soil matrices at the same time point. These preliminary findings indicate that MAD reduces the number of eggs and potential environmental release; however, the study is ongoing and will continue through day 180

(September) to determine its capacity for complete elimination. If these results are maintained, biodigesters with MAD systems could represent a safe alternative for controlling *T. solium* transmission.

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SURGICAL MANAGEMENT OF HEPATIC CYSTIC ECHINOCOCCOSIS: A SINGLE-CENTER EXPERIENCE FROM A WHO COLLABORATING CENTER

Ottavia Cicerone[1], **Raffaella Lissandrin**[2], Ambra Vola[2], Leka Armanda[2], Pietro Rinaldi[3], Enrico M. Brunetti[4], Marcello Maestri[2]

[1]University of Pavia, Pavia, Italy, [2]Foundation IRCCS Policlinico San Matteo, Pavia, Italy, [3]Alessandria, AL, Italy, [4]University of Pavia, San Matteo Hospital Foundation, Pavia, Italy

Cystic echinococcosis (CE) is a zoonotic parasitic disease with an expanding global distribution driven by migration and population mobility, increasingly encountered in non-endemic settings. The liver is the most frequently involved organ, and management requires a stage-adapted, multidisciplinary approach. We retrospectively analyzed patients undergoing surgery for hepatic CE at a WHO Collaborating Center between January 2009 and December 2025, evaluating indications, surgical strategies, and outcomes. Among 417 patients diagnosed with hepatic CE, 94 (22.5%) underwent surgery after multidisciplinary assessment. Most patients were symptomatic or presented high-risk imaging features, including risk of rupture, extensive hepatic involvement, or biliary or vascular compression. Transitional cysts (CE3a-CE3b) represented most cases (78.7%). Surgical management prioritized parenchyma-sparing techniques, with combined endocystectomy and partial pericystectomy and total pericystectomy being the most frequently adopted procedures. Minimally invasive approaches were applied in highly selected cases (2.1%), while combined or staged thoracic surgery was required in patients with concomitant pulmonary disease. No postoperative mortality was observed. Major complications occurred in 10.6% of patients, mainly biliary fistulas and intra-abdominal collections managed with endoscopic or percutaneous interventions. During follow-up, recurrence occurred in three patients, confirming durable disease control. These findings support surgery as a safe and effective treatment when appropriately indicated and performed in specialized centers. A tailored, stage-adapted strategy is essential to avoid both overtreatment and undertreatment. Parenchyma-sparing approaches should be prioritized, while minimally invasive surgery may be considered in selected cases with favorable characteristics. Given the evolving epidemiology of CE, multidisciplinary management is essential.

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RISK FACTORS FOR BREAKTHROUGH SEIZURES IN PATIENTS WITH ACTIVE EPILEPSY DUE TO CALCIFIED NEUROCYSTICERCOSIS (NCC)

Carolina Guzman[1], Selene Salas[2], Oscar H. Del Brutto[3], Gianfranco Arroyo[2], Sofia Sanchez[4], Robert H. Gilman[5], Herbert Saavedra[6], Isidro Gonzales[6], Seth O'Neal[7], Cilene Ramirez[2], Marco Vasquez[2], Hector H. Garcia[8], Javier A. Bustos[9]

[1]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [2]Universidad Peruana Cayetano Heredia, Lima, Peru, [3]Dr. Oscar Del Brutto, Samborondon, Ecuador, [4]Center for Global Health, [5]Johns Hopkins Univ Bloomberg School of Public Health, Baltimore, MD, United States, [6]Instituto Nacional de Ciencias Neurológicas, Lima, Peru, [7]Oregon Health & Sciences University, Portland, OR, United States, [8]Univ Peruana Cayetano Heredia, Lima, Peru, [9]Universidad Peruana Cayetano Heredia, LIMA, Peru

Calcified neurocysticercosis (NCC) can continue to affect patients long after the infection has resolved. Symptoms, mainly seizures and headaches, depend on individual risk factors such as lesion number, location, host immune response and others. Taking advantage of a large cohort of individuals with calcified NCC, we aimed to determine the incidence and risk factors for breakthrough seizures (BTS) in patients with epilepsy secondary to calcified NCC. A subgroup of patients from our calcified NCC cohort who met criteria for epilepsy and were receiving antiepileptic treatment underwent clinical examination, electroencephalography (EEG), and enzyme-linked immunoelectrotransfer blot (EITB) testing, and were prospectively followed until the occurrence of BTS, AED withdrawal, loss to follow-up, or administrative censoring at 4 years. A total of 316 patients (mean age 38 years; 52% female, mean duration of epilepsy prior to enrollment 59.5 months) met inclusion criteria. During follow-up, 159 patients (50.3%) experienced breakthrough seizures (BTS). Most had focal start (78.5%) followed by 21.5% who had generalized seizures. 55% had experienced more than 10 lifetime seizures. Most participants had weak positive EITB (1-3 bands, 47%) or were seronegative. Most participants had previously received antiparasitic treatment (67%) and had more than one calcified lesion on CT (58%).On univariate Cox regression female participants, those with seizures in the year before enrollment, and those with more than 10 lifetime seizures had a higher risk of BTS. On multivariate analysis, seizures within the past 12 months (HR 2.11, $p < 0.001$) and having more than 10 lifetime seizures (HR 1.43, $p = 0.033$) were independent risk factors for BTS. In conclusion, approximately half of individuals with calcified NCC and a history of epilepsy experienced BTS over four years of follow-up. The strongest independent risk factor for BTS was having active seizures within the year prior to enrollment.

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NEURONAL AND SYNAPTIC STRUCTURAL CHANGES ASSOCIATED WITH COGNITIVE DEFICITS ASSESSED USING THE T-MAZE IN AN ANIMAL MODEL OF NEUROCYSTICERCOSIS

Solange B. Custodio Custodio[1], Danitza Griselda Dávila-Villacorta[2], Laura Estefania Baquedano Santana[3], Edson Gustavo Bernal Teran[1], Robert H. Gilman[4], Manuela Renee Verastegui[1]

[1]Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Departamento de Medicina Preventiva y Salud Pública, Facultad de Medicina. Universidad Nacional Mayor de San Marcos, Lima, Perú, Lima, Peru, [3]Universidad Peruana Cayetano Heredia, Bethel Park, PA, United States, [4]Johns Hopkins Univ Bloomberg School of Public Health, Baltimore, MD, United States

Neurocysticercosis (NCC) is a parasitic disease associated with epilepsy and cognitiveimpairment, and research on this condition in humans is limited. An animal model of NCC wasused in rats infected with activated oncosphères of *Taenia solium* to analyze axonal and synapticdamage and its possible association with memory deficits, as assessed using the T-Maze test 12months post-infection. A total of 101 brain samples (65 infected and 36 healthy) were analyzed,revealing that neurofilament and synaptophysin exhibited spheroidal accumulation inperilesional regions in infected animals, indicating neuronal damage at the axonal level andsynaptic vesicle disruption, respectively. Likewise, a significant decrease in the percentage ofcorrect responses on the T-Maze test was observed in infected animals compared to controls.Based on this, an inverse correlation was found between protein accumulation and memoryperformance, demonstrating that greater neuronal structural and synaptic damage wasassociated with a lower success rate, suggesting that disruption of neuronal integrity andsynaptic connectivity contributes to cognitive impairment in neurocysticercosis. In this context,it is important to explore compensatory mechanisms such as adult neurogenesis that may playa role in the response to damage and functional recovery, providing new insights into theinteraction between degeneration and repair in this disease.

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TEMPORAL CHARACTERIZATION OF MICROGLIAL ACTIVATION IN AN ANIMAL MODEL OF NEUROCYSTICERCOSIS

Danitza Griselda Dávila-Villacorta[1], Rensson Céliz-Ygnacio[2], Fabio César Torres-Bocanegra[3], César Miguel Gavídia[4], Robert H. Gilman[5], Manuela Renee Verástegui-Pimentel[6]

[1]Departamento de Medicina Preventiva y Salud Pública, Facultad de Medicina, Universidad Nacional Mayor de San Marcos, Lima, Peru, [2]Universidad Científica del Sur, Lima, Peru, [3]Universidad Nacional Federico Villarreal, Lima, Peru, [4]School of Veterinary Medicine, Universidad

Nacional Mayor de San Marcos, Lima, Perú, Lima, Peru, [5]Johns Hopkins Univ Bloomberg School of Public Health, Baltimore, MD, United States, [6]Universidad Peruana Cayetano Heredia, Lima, Peru

Neurocysticercosis (NCC) is a parasitic disease of the central nervous system in which the host inflammatory response plays a key role in pathogenesis. In this study, the kinetics of microglial activation were evaluated in an animal model of NCC across post-infection time points (1, 1.5, 3, 6, and 12 months post infection). Immunoreactivity for CD68, a marker of activated microglia, showed high reactivity at all evaluated time points. However, a progressive change in microglial morphology was observed, suggesting the presence of different microglial populations over the course of infection: early stages (1 - 1.5 months) were characterized by predominantly amoeboid morphology, intermediate stages (3-6 months) showed hypertrophic and dystrophic microglia, and late stages (12 months) were predominantly characterized by de-ramified dystrophic morphology. These findings indicate a temporal dynamic in microglial activation. Ongoing analyses include the evaluation of markers associated with microglial polarization, including CD16 and CD32 (M1) and CD206 and CD163 (M2). It is expected that early stages will show predominance of the M1 phenotype, associated with a pro-inflammatory response, while later stages will show predominance of the M2 phenotype, associated with an anti-inflammatory response. This hypothesis is supported by prior observations of fibrosis using picrosirius red staining, where no fibrosis was observed in early time points, whereas increased fibrosis was detected at later stages. These findings will contribute to understanding the temporal evolution of microglial responses in NCC and their potential association with inflammatory and fibrotic processes during disease progression.

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ARTIFICIAL INTELLIGENCE IN THE IMAGING DIAGNOSIS OF CYSTIC ECHINOCOCCOSIS: A SCOPING REVIEW

Saul Javier Santivanez[1], Oswaldo Gabriel Ernesto Espinoza-Hurtado[2], Raul Enriquez-Laurente[3]

[1]Universidad Señor de Sipan, Chiclayo, Peru, [2]Universidad Continental, Huancayo, Peru, [3]Universidad Continental, Puente Piedra, Peru

Cystic echinococcosis (CE) is a neglected zoonotic disease with substantial clinical and socioeconomic burden, particularly in endemic regions. Imaging plays a central role in diagnosis and staging, yet its accuracy depends heavily on expert interpretation, leading to variability in clinical decision-making. Artificial intelligence (AI) has emerged as a promising tool to enhance imaging diagnostics. This study aimed to map the current evidence on AI applications in the imaging diagnosis of CE and evaluate their diagnostic performance. A scoping review was conducted following PRISMA-ScR guidelines . A comprehensive search of PubMed/MEDLINE, Scopus, IEEE Xplore, and Google Scholar identified studies evaluating AI-based methods applied to CE imaging. Eligible studies included original research on machine learning (ML), deep learning (DL), or computer-aided diagnostic systems using ultrasound (US), computed tomography (CT), or magnetic resonance imaging (MRI). Data extraction captured study characteristics, imaging modalities, AI architectures, and diagnostic performance metrics. A total of 20 studies were included from 822 records screened . Most studies were published between 2019 and 2025 and predominantly used ultrasound (~70%), followed by CT and MRI. There has been a clear shift from traditional ML models to advanced DL architectures, particularly convolutional neural networks and hybrid models. Reported diagnostic performance was consistently high, with accuracies exceeding 90% and reaching up to 97.5% for CT-based models and 100% in controlled MRI datasets. AI systems demonstrated high sensitivity (>95%) in identifying active and inactive CE stages, though classification of transitional stages (CE3) remains challenging. AI demonstrates strong potential to standardize CE imaging diagnosis and improve access to high-quality interpretation, especially in resource-limited settings. Future research should prioritize multicenter validation, integration of clinical data, and prospective studies assessing real-world impact on patient outcomes.

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PERFORMANCE OF FLOTATION METHODS FOR RECOVERING *TAENIA SOLIUM* EGGS FROM SOIL USING MICROSCOPY AND QPCR

Daniel A. Andrade[1], Cesar A. Valdivia-Carrera[2], Sophie De Bock[3], Brenda L. Gonzales[2], Ana C. Ho-Palma[4], Sarah Gabriël[3], Luis A. Gomez-Puerta[2], Javier A. Bustos[1], Seth E. O'Neal[5], Hector H. Garcia[1], Eloy Gonzales-Gustavson[2]

[1]Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Universidad Nacional Mayor de San Marcos, Lima, Peru, [3]Ghent University, Ghent, Belgium, [4]Universidad Nacional del Centro del Perú, Huancayo, Peru, [5]Oregon Health & Sciences University, Portland, OR, United States

Understanding the survival and dispersal of *Taenia solium* eggs in the environment is critical for elucidating transmission and informing control strategies. However, their recovery from soil is limited by matrix effects and methodological constraints. We conducted a factorial experiment to evaluate three flotation methods (saturated sugar, saturated salt-sugar, and zinc chloride) across three soil types (loam, clay, silt) and two egg concentrations: high (50 EPG) and low (2 EPG). Experiments were performed in triplicate using 50 g of spiked soil per sample. Egg recovery was assessed immediately after processing by microscopy and within one week by real-time PCR (qPCR). At 50 EPG, microscopy-based recovery was significantly influenced by the interaction between method and soil type ($p = 0.0028$). The saturated sugar method achieved the highest recovery (18.9% in loam; 14.5% in clay; 9.3% in silt), compared to salt-sugar (8.7%, 5.1%, and 2.6%) and zinc (≤2.6% across soils), with a significant decrease in recovery in silt. No eggs were detected at 2 EPG, highlighting a critical sensitivity threshold. In contrast, qPCR detected *T. solium* DNA at both concentrations. At 50 EPG, the sugar method yielded the highest recovery (39.7%-56.8%), followed by salt-sugar (11.2%-37.7%) and zinc (22.8%-51.8%), although zinc showed high variability. At 2 EPG, qPCR detected DNA across all conditions, with lower recovery (0.46%-20.4%). A strong positive correlation was observed between genomic copy number and microscopy-based egg counts (Spearman's $\rho = 0.90$, $p < 0.001$), supporting qPCR as a proxy for egg burden. However, qPCR cannot distinguish between viable eggs and free extracellular DNA, which may lead to overestimation of environmental contamination. Current concentration methods remain inadequate for low-level contamination. While qPCR is a superior tool for sensitive detection, a positive qPCR does not confirm the presence of viable eggs. Improvements in recovery techniques are essential to enable both microscopy-based identification and formal viability assessments in field conditions.

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THE *TAENIA SOLIUM* NOVEL PA1-LIKE PROTEIN REMODELS GLUCOSE METABOLISM BY DISRUPTING THE MTORC1 SIGNALING AND TILTS MACROPHAGES TOWARDS IMMUNE SILENCE

Parul Mehra, Amit Prasad

Indian Institute of Technology Mandi, Mandi, India

Neurocysticercosis, a neurological helminth infection caused by *Taenia solium* is a significant contributor to acquired epilepsy, accounting for approximately 30% cases in endemic regions. *T. solium* excretory secretory proteome (ESP), the key mediator between the helminth and host immune system, remains poorly characterized despite containing biologically relevant molecules. Our proteomic analysis of this ESP identified 247 proteins, which span across both pro- and anti-inflammatory functions. Among these, we identified and extensively characterized a novel molecule, the PA1. Our findings demonstrate efficient internalization of PA1 in differentiated THP-1 macrophages leading to significant alterations in cellular metabolism. PA1 decreases the intracellular levels of glucose and downregulates the expression level of glucose transporters, GLUT1 and GLUT3. This metabolic reprogramming is associated to disruption in the mTORC1 signaling. Further, this signaling cascade reduces the oxidative stress in macrophages and inclines the immune response towards an

anti-inflammatory phenotype. Additional experimental and bioinformatic analysis of PA1 confirm its stability over molecular dynamics simulations. Collectively, PA1 represents a key parasitic molecule which can specifically rewire cellular metabolism and influence the immune response to *T. solium* infection providing a compelling avenue for elucidating host-parasite interactions and developing potential therapeutic strategies.

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SPECIFIC PARASITE ANTIGEN INTERPLAY REGULATES MONOCYTE-DRIVEN NEUROINFLAMMATION IN NEUROCYSTICERCOSIS

Luz M. Toribio[1], Deborah L. Chong[2], Hector H. Garcia[1], Jon Friedland[3]

[1]Universidad Peruana Cayetano Heredia, Lima, Peru, [2]City St.George's University of London, London, United Kingdom, [3]City St George's University of London, London, United Kingdom

Neurocysticercosis (NCC), caused by *Taenia solium* infection of the brain, is characterized by intense inflammation driven by a diversity of parasite antigens. While some antigens have been linked to inflammatory responses, it remains unknown how antigen combinations interact to modulate monocyte activation. We investigated how recombinant diagnostic antigens (rT24H, rGP50, 8kDa) affect monocyte activation and downstream astrocyte polarization. Human monocytes were stimulated with individual or combined antigens for 48 h. Secretion of TNF-α, IL-1β, and IL-6 were measured by ELISA. To investigate their influence on astrocyte polarization, primary human astrocytes (HA) were stimulated with conditioned media from stimulated monocytes and changes in gene expression of astrocyte phenotype biomarkers (c3/ip10 for A1-neurotoxic and s100a/vegfa for A2-neuroprotective phenotype) were assessed at 48 h post-stimulation. rT24H induced robust inflammatory cytokine production of TNF-α and IL-1β, whereas rGP50 elicited weaker responses (TNF-α=18089.45 vs 355.72, IL-1β=1283.33 vs 182.95 pg/ml). 8kDa did not elicit any inflammatory responses. Antigen combinations produced different effects: rT24H+8kDa markedly enhanced cytokine secretion beyond rT24H alone (TNF-α=21355.58, IL-1β=2133.5, IL-6=5088.62pg/ml), while rGP50+rT24H reduced TNF-α and IL-1β in 10.8 and 5.15-fold changes, respectively; and maintaining IL-6, suggesting a modulatory effect of rGP50. rGP50+8kDa resulted in minimal cytokine induction. HA experiments showed that only HA exposed to rT24H+8kDa-conditioned media exhibited a significant shift toward a neurotoxic A1 phenotype, characterized by increased c3 and IP-10 gene expression (78.6-fold and 96.8-fold compared to unstimulated HA), whereas non-significant changes were observed for A2 genes in any of conditions. Altogether these findings demonstrate that antigen interactions can both suppress and amplify monocyte-driven inflammation with downstream consequences for astrocyte activation. Antigen composition may therefore be a key determinant of inflammatory NCC heterogeneity.

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LOW FUNCTIONAL ANTIBODIES TO *PLASMODIUM FALCIPARUM* ANTIGENS IN BENINESE NEONATES FURTHER REDUCED THE TIME TO FIRST MALARIA INFECTION IN INFANCY FOLLOWING PLACENTAL MALARIA

Djelili Biaou[1], Sebastien Dechavane[1], Kana Ikhlaq Hussain[2], Aziz Bouraïma[3], Ibrahim Sadissou[3], André Garcia[1], Florence Migot-Nabias[1], Ed Remarque[2], Jafari-Guemouri Sayeh[1], Gineau Laure[1], David Courtin[4], Achille Massougbodji[5], Michael Theisen[2], Felix Cheysson[6], Celia Dechavane[1]

[1]Université Paris Cité, IRD, Inserm, MERIT, F-75006 Paris, France, Paris, France, [2]Centre for Medical Parasitology at Department of International Health, Immunology and Microbiology, University of Copenhagen and Department for Congenital Disorders, Statens Serum Institute, Copenhagen, Denmark, [3]CERPAGE (Centre d'Etude et de Recherche sur les Pathologies Associées à la Grossesse et à l'Enfance), Cotonou, Benin, [4]UMR 177 INTERTRYP, IRD CIRAD and Université de Montpellier, Montpellier, France, [5]Institut de Recherche Clinique du Bénin (IRCB), Cotonou, Benin, [6]LAMA (Laboratoire d'analyse et de mathématiques appliquées), Paris, France

The transplacental transfer of maternal antibodies to the fetus is crucial in protecting neonates in early life. However, several studies have shown that infants from mothers with Malaria infection at Delivery (MiD) present a high risk of infection and constitute a particularly vulnerable population. Many studies have investigated the levels of transferred maternal antibodies in MiD whereas the functionality of those antibodies remains to be assessed. Moreover, the capability of these functional antibodies to protect from malaria infection during infancy remains unknown. This study was carried out in southern Benin and neonates were followed up from birth to 18 months of age (n=561). The levels of immunoglobulin G (IgG), IgG1 and IgG3 specific to four *Plasmodium falciparum*-antigen were quantified ELISA. The functionality of IgG against same four *Pf*-antigens was determined with a bead-based opsonic phagocytosis assay (BPA) using THP1 monocyte line and 355 paired maternal and cord serums. Placental malaria was associated with increased IgG (Coef = 0.47, [0.12;0.82], p =0.007) and subclass IgG1 and IgG3 levels without improving antibody function. Placental malaria and hypergammaglobulinemia reduced transplacental transfer of specific IgG (Coef = -0.15, [-0.26;-0.05], p =0.003) resulting in lower fetal IgG concentrations at birth (Coef = -0.17, [-0.27;-0.07], p =0.001). Moreover, functional IgG transfer was impaired by placental malaria (Coef = -0.11, [-0.23;-0.0003], p = 0.04). In our study, fetal opsonic phagocytosis decreased in case of placental malaria and maternal hypergammaglobulinemia. In infants, higher concentrations and greater functionality of IgG were associated with protection up to 351 and 208 days, respectively. Infants born to mother with placental malaria and low levels of functional fetal IgG had a significantly higher risk of the first malaria compared to those with highly functional IgG and born to mother without placental malaria (HR = 7.90, 95% CI: 3.66–17.05, p < 0.001). Overall, our study showed that protection of infant from first malaria infection involved multiple mechanisms, including IgG-mediated phagocytosis.

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SEASONAL MALARIA CHEMOPREVENTION REMODELS THE BLOOD TRANSCRIPTOME IN UGANDAN CHILDREN

Jane Achan[1], Yoweri Taremwa[2], Caroline Duncombe[2], Stephen Tukwasibwe[3], Richard Kajubi[4], Kenneth Musinguzi[3], Asadu Sserwanga[4], Anthony Nuwa[4], Isaac Ssewanyana[3], James Kananura Tibenderana[1], Prasanna Jagannathan[2]

[1]Malaria Consortium, London, United Kingdom, [2]Stanford University, Stanford, CA, United States, [3]Central Public Health Laboratories, Kampala, Uganda, [4]Malaria Consortium, Kampala, Uganda

The impact of seasonal malaria chemoprevention (SMC) on the development of naturally acquired immunity remains a critical knowledge gap. While SMC reduces clinical episodes, resulting reduction in parasite exposure may remodel the immune system. We characterized host blood transcriptomes in children across varying historical SMC exposure to understand long-term immunological trade-offs. A prospective cohort study was done during the 2024 SMC season in three Ugandan districts with divergent SMC history: Moroto (>3 prior rounds), Napak (>2 prior rounds), and Katakwi (SMC-naive). We enrolled 196 children (3-59 months) and did RNA sequencing on whole blood collected at baseline (pre-SMC) and endline (post-SMC). Differential gene expression (DEG) and pathway enrichment via blood transcriptional modules (BTMs) were analyzed, adjusting for age, sex, and asymptomatic infection status. At baseline, children with prior SMC exposure exhibited a distinct pro-inflammatory signature compared to SMC-naive children, characterized by significant upregulation of myeloid/monocyte modules, neutrophils, and TLR signaling. Conversely, SMC-naive children showed a signature dominated by B cell-mediated adaptive immunity. Following a single SMC round, these differences intensified. Moroto children showed the most robust transcriptomic shift, with marked upregulation of NK and T cell modules. By endline, the divergence reached its peak: Napak children showed massive upregulation of inflammatory pathways (including IL-1B) compared to Katakwi. Notably, B cell modules remained consistently suppressed in all

SMC-exposed groups across both timepoints, suggesting a persistent shift away from adaptive priming. Our findings demonstrate that repeated SMC rounds progressively remodel the pediatric immune landscape, shifting responses from adaptive B cell-mediated pathways toward a more innate, inflammatory state. The results suggest that while SMC provides essential protection, it may fundamentally alter the trajectory of clinical immunity. These immunological trade-offs are vital for policymakers to consider as SMC programs expand.

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PARASITE DENSITY AND CYTOKINE RESPONSES IN MALARIA: DECOUPLING PARASITAEMIA FROM IMMUNE ACTIVATION IN SOUTHERN NIGERIA

Ginika Lovelyn Onwuachusi[1], Chinyelu Angela Ekwunife[1], Wellington Aghoghovwia OYIBO[2]

[1]Department of Parasitology and Entomology, Nnamdi Azikiwe University, Awka, Nigeria, [2]Centre for Transdisciplinary Research for Malaria and Neglected Tropical Diseases (CENTRAL-NTD)/ANDI Centre of Excellence for Malaria Diagnosis, College of Medicine, University of Lagos, Lagos, Nigeria

The relationship between malaria parasite density and immune activation remains incompletely understood. While parasitaemia is often assumed to drive proportional inflammatory responses, emerging evidence suggests a potential decoupling between parasite burden and immune activation, particularly in malaria-endemic populations. This study investigated the relationship between malaria parasitaemia and cytokine responses in symptomatic and asymptomatic individuals in Southern Nigeria. A cross-sectional study was conducted among 544 participants recruited from Lagos and Anambra States, Nigeria. A subset of 174 samples (symptomatic = 128; asymptomatic =46), including microscopy confirmed positive (n=96) and Microscopy negative controls (n=78), underwent immunological profiling; plasma levels of IgG, TNF-α, and IL-8 were quantified using ELISA. Parasite density was categorised into low (≤1,000), medium (1,001-10,000), and high (>10,000 parasites/μL). Statistical analyses included non-parametric tests, correlation analysis and linear regression. Symptomatic individuals had significantly higher parasite densities (GMPD = 8,009 parasites/μL) than asymptomatic individuals (GMPD = 953 parasites/μL; $p < 0.001$). However, TNF-α and IL-8 levels did not differ significantly across parasite density groups. Regression analysis demonstrated weak associations between parasitaemia and cytokine levels (Symptomatic: TNF-α R^2=0.001, IL-8 R^2= 0.002; Asymptomatic: TNF-α R^2 =0.082, IL-8 R^2= 0.103). IgG responses also showed no consistent association with parasite density with cytokine responses remaining relatively stable despite variations in parasitaemia. Parasitaemia did not predict cytokine or antibody responses, demonstrating a functional decoupling between parasite burden and immune activation. These findings reinforce the role of immune regulation and tolerance in malaria-endemic population and highlight limitations of using parasitaemia alone as a measure of cytokine activation.

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BALANCED IMMUNITY, PERSISTENT PARASITES: IMMUNE FEATURES OF ASYMPTOMATIC *PLASMODIUM VIVAX* INFECTIONS IN THE BRAZILIAN AMAZON

Gregorio Guilherme Almeida[1], Camila Medeiros Costa[1], Pedro Augusto Carvalho Costa[1], Maisa da Silva Araujo[2], Gabriela Ribeiro Gomes[1], Maria Marta Figueiredo[3], Katherine Jéssica Torres[4], Alex Fiorini Carvalho[5], Bruno Vinícius Santos Valiate[5], Julia Ramos Sampaio[5], Camila Rodrigues Barbosa[6], Brener Cunha Carvalho[1], Dhelio Batista Pereira[7], Alexia Martines[7], Mauro Shugiro Tada[7], Irene Silva Soares[8], Flora S. Kano[1], Luzia H. Carvalho[1], Jamie Ponmattan[9], Marcia Caldas Castro[9], Joseph M. Vinetz[10], Douglas T. Golenbock[11], Caroline Junqueira[1], Ricardo Gazzinelli[11], Lis Ribeiro do Valle Antonelli[1]

[1]Fundação Oswaldo Cruz - Instituto René Rachou, Belo Horizonte, Brazil, [2]Fundação Oswaldo Cruz - Fiocruz Rondônia, Porto Velho, Brazil, [3]Universidade Estadual de Minas Gerais, Belo Horizonte, Brazil, [4]Universidad Peruana Cayetano Heredia, Lima, Peru, [5]CT Vacinas - Universidade Federal de Minas Gerais, Belo Horizonte, Brazil, [6]Oswaldo Cruz Foundation, Belo Horizonte, Brazil, [7]Centro de Pesquisa em Medicina Tropical, Porto Velho, Brazil, [8]Universidade de São Paulo, São Paulo, Brazil, [9]Harvard T.H. Chan School of Public Health, Boston, MA, United States, [10]Yale University, New Haven, CT, United States, [11]University of Massachusetts Medical School, Worcester, MA, United States

Individuals with asymptomatic *Plasmodium vivax* infection are considered important reservoirs sustaining malaria transmission in endemic regions. We investigated both the transmission potential and the adaptive immune responses associated with asymptomatic infection in a cohort from an urban area of the Brazilian Amazon. Functional transmission assays demonstrated that blood from asymptomatic individuals, even with very low parasitemia, could infect the primary local vector, *Anopheles darlingi*, although at lower rates compared to symptomatic patients. Notably, several asymptomatic individuals sustained parasitemia over extended periods, reinforcing their role as persistent infectious reservoirs. To better understand the immunological basis of this clinical state, we performed an integrative analysis of the adaptive immune compartment. Our integrative analysis revealed a T_H1-biased immune signature with expanded populations of T_H1 $CD4^+$ T cells associated with the asymptomatic infection. In addition, there is an expanded population of proliferating atypical memory B cells that correlates with IgG levels against *P. vivax* antigens and parasitemia. The absence of systemic inflammation based on a comprehensive panel of soluble markers and the lower expression of some regulatory markers suggests a controlled inflammatory response that can be derived from an effective control of parasite growth. Our findings suggest that ASY maintains a pool of IFN-γ-associated Th cell phenotypes that orchestrate the immune response, limiting parasitemia and preventing clinical malaria.

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NEW INSIGHTS INTO THE ACQUISITION OF FUNCTIONAL ANTIBODY RESPONSES AGAINST *PLASMODIUM KNOWLESI* AND *PLASMODIUM VIVAX* MALARIA

Kaitlin Pekin[1], Jill Chmielewski[2], Isabelle Henshall[2], Jarryd Tiu[2], Rajahram S. Giri[3], Timothy William[4], Sitti Saimah Sakam[5], Daniel J. Cooper[6], Bridget Barber[7], Matthew Grigg[7], Nick Anstey[7], Danny Wilson[2], D. Herbert Opi[1], James Beeson[1]

[1]Burnet Institute, Melbourne, Australia, [2]Adelaide University, Adelaide, Australia, [3]Queen Elizabeth II Hospital, Kota Kinabalu, Sabah, Malaysia, [4]Queen Elizabeth Hospital II, Kota Kinabalu, Sabah, Malaysia, [5]Infectious Diseases Society Sabah Menzies, Kota Kinabalu, Sabah, Malaysia, [6]University of Cambridge, Cambridge, United Kingdom, [7]Menzies School of Health Research, Darwin, Australia

Outside of Africa, multiple species contribute to malaria burden, including *Plasmodium falciparum, P. vivax* and *P. knowlesi. P. knowlesi,* is a zoonotic infection with a macaque reservoir throughout Asia, complicating elimination. In Malaysia, as *P. falciparum* and *P. vivax* cases decrease, *P. knowlesi* is now the leading cause of malaria and prioritised for elimination. Despite growing prevalence, there is a limited understanding of protective immunity or the mechanisms involved. We used culture adapted *P. knowlesi* to investigate the development of functional immunity to *P. knowlesi* and closely related *P. vivax*. In a cohort of *P. knowlesi* exposed individuals from Sabah, Malaysia, we measured functional antibody responses against *P. knowlesi* merozoites. High levels of IgG and IgM to intact merozoites among *P. knowlesi* exposed individuals. Merozoites were targets of functional antibody responses including Fcγ receptor (FcγRI, II and IIIa) binding and complement fixation, functions that have been linked to *P. falciparum* immunity, with responses varying between individuals. We observed high levels of antibody binding to three Fcγ receptors, each strongly correlated with IgG1. Interestingly, IgG2 strongly correlated with FcγRII binding. Additionally, due to high levels of homology between *P. knowlesi* and *P. vivax*, we investigated cross-reactive antibodies in a naturally exposed *P. vivax* cohort from Papua New Guinea, with no prior *P. knowlesi* exposure. We observed high levels of cross-reactive antibodies to *P. knowlesi* merozoites including IgG, Fcγ receptor binding and complement

fixation. Studies with culture adapted *P. knowlesi* revealed new insights into immunity against relatively unknown species: *P. knowlesi* and *P. vivax.* Individuals exposed to *P. knowlesi* and cross-reactive antibodies from *P. vivax* exposed individuals, mediated a range of potent functional antibody responses against *P. knowlesi* merozoites. A vaccine, that induces high levels of cross-reactive antibodies to *P. vivax* and *P. knowlesi,* may be achievable to support in elimination of both species.

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IDENTIFYING IMMUNE CORRELATES OF PROTECTION TO *PLAMODIUM VIVAX* USING HUMAN MONOCLONAL ANTIBODIES TO APICAL MEMBRANE ANTIGEN 1

Anna C. Winnicki, Alyssa N. Shepard, Lenore L. Carias, Yelenna Skomorovska-Prokvolit, Fadia Naqash, Michael Payne, Christopher L. King

Case Western Reserve University, Cleveland, OH, United States

One-third of the human population, mainly in South America and Southeast Asia, is at risk of contracting *Plamodium vivax* (Pv) malaria. Apical Membrane Antigen 1 (AMA1) is a critical surface protein that binds to Rhoptry Neck Protein 2 (RON2); this interaction is utilized by both sporozoites and merozoites and is vital for invading liver cells and reticulocytes, respectively. PvAMA1 is a highly immunogenic surface antigen, and individuals with high antibody (Ab) levels to AMA1 are associated with a decreased risk of malaria. We have identified two PvAMA1-specific human monoclonal antibodies (humAbs), 826827 and 864865, that target epitopes on Domain 1 of AMA1 and inhibit Pv infection both in vitro and in vivo, with cross-strain blocking effects. Competitive ELISA using these humAbs shows that a certain percentage (6 to 73%) of individuals from various Pv-endemic areas develop blocking antibodies, ranging from 33.3 to 97% blocking of 826827 and from 36 to 99% blocking of 864865. Children under 12 demonstrate more blocking of 826827 compared to 864865, while adults exhibit greater blocking of 864865. We hypothesize that individuals with the highest levels of blocking antibodies against these epitopes have a lower risk of Pv infection and disease. These analyses are ongoing through our well-established longitudinal cohort studies of Pv in Papua New Guinea, the Solomon Islands, and Brazil. These findings will support the use of these humAbs for preventative therapy to stop Pv infection and aid in developing a Pv vaccine that includes these epitopes, thus targeting both pre-erythrocytic and blood-stage infections.

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STUDYING THE ANTIBODY RESPONSE TO THE PCRCR COMPLEX IN MALARIA INFECTION

Andrew Cooper[1], Joshua Tan[2]

[1]NIAID NIH, Rockville, MD, United States, [2]National Institutes of Health, Rockville, MD, United States

A major goal in generating new interventions against malaria is the development of vaccines or monoclonal antibodies that strongly inhibit the blood stage of the *Plasmodium falciparum* life cycle. The PCRCR complex is composed of five antigens (PTRAMP, CSS, Ripr, CyRPA and RH5) that are leading blood-stage malaria vaccine candidates, as all five components are highly conserved and essential for RBC invasion. However, the mechanism of antibody function against this complex is still poorly understood. To address this question, we isolated a combined total of 273 mAbs to RH5 and CyRPA from malaria-exposed individuals. We identified clear predictors of neutralization potency for these two mAbs, including binding affinity and target epitope. All neutralizing mAbs against RH5 bound at the 'top' of RH5, closest to where it binds the host protein basigin. Work to evaluate the functioning of mAbs against CyRPA, Ripr, CSS and PTRAMP remains ongoing. Most mAbs from malaria-exposed individuals were non-neutralizing, but a small number of infection-derived mAbs showed similar potency to the current best-in-class vaccine-derived PCRCR mAb. Taken together, our findings demonstrate that exposure to *P. falciparum* can elicit rare, potent mAbs against PCRCR, and inform the development of epitope-focused next-generation vaccines.

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SYSTEMS SEROLOGY INSIGHTS INTO THE KINETICS AND DYNAMICS OF GROWTH-INHIBITORY IMMUNE RESPONSES TO *PLASMODIUM FALCIPARUM* STRAINS

Kelly A. Hagadorn[1], Mouhamad Sy[2], Awa Bineta Deme[3], Ibrahima M. Ndiaye[3], Younous Diedhiou[3], Amadou M. Mbaye[3], Qin Xiao[4], Zizhang Sheng[4], Kate Levine[5], Ross Blanc[5], Qixin Wang[5], Sarah K. Volkman[5], Carole A. Long[6], Kazutoyo Miura[7], Ababacar Diouf[6], Ryan P. McNamara[5], Daouda NDIAYE[2], Amy Kristine Bei[1]

[1]Department of Epidemiology of Microbial Diseases, Yale School of Public Health, New Haven, CT, United States, [2]Laboratory of Parasitology and Mycology, Cheikh Anta Diop University, Aristide le Dantec Hospital, Dakar, Senegal, [3]International Research and Training Center for Applied Genomics and Health Surveillance (CIGASS) at UCAD, Dakar, Senegal, [4]Division of Infectious Diseases, Department of Medicine, Columbia University Vagelos College of Physicians and Surgeons, New York, NY, United States, [5]Department of Immunology and Infectious Diseases, Harvard T. H. Chan School of Public Health, Boston, MA, United States, [6]Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States, [7]Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States

The identification of conserved *Plasmodium falciparum* antigenic targets that are true correlates of immune protection would transform malaria immunology and vaccinology but requires a deeper understanding of the mechanisms of naturally acquired functional immunity. We performed 51 *in vitro* growth inhibition assays (GIA) using field-isolated and adapted *P. falciparum* isolates and evaluated merozoite antigen–specific antibody recognition and biophysical features to understand functional immune kinetics and dynamics over time. Patients in a longitudinal cohort in Thiès, Senegal (n=70; low-endemicity, mostly monogenomic infections) were followed for 2 years after initial infection, with plasma collected at day 0; weeks 2 and 4; and months 3, 6, 12, 18, and 24. Parasite isolates from day 0 were culture adapted (n=66) and GIAs were performed with matched day 0 isolates (n=51) using purified IgG from all follow-up timepoints (n=428). Three main inhibitory antibody patterns were observed across the 2 year follow-up: long-persisting high responses, early peak (week 2) with rapid decline (month 3), and negligible responses. A multiplexed systems serology approach for 26 merozoite antigens assessed eight antigen specific antibody biophysical features (total IgG, IgG1, IgG3, IgM, FcγRIIa, FcγRIIb, FcγRIIIa, FcγRIIIb) of naturally acquired high inhibitory responses. IgG1-specific CyRPA, EBA175, GAMA, MSP11, MSP3, and MTRAP and IgG3-specific EBA140, EBA175, and MSP11 antibodies correlated higher GIA at day 0 and month 3 ($p<0.05$, $rho>0.4$). Unsupervised and supervised analyses are ongoing to define antibody biophysical features that sustain inhibition and reveal novel insights into protective immunity. Together, these data increase our understanding of natural immunity by identifying sustained antigen-specific antibodies important for inhibitory responses that can be leveraged for next-generation vaccines and immune-monitoring tools to accelerate development of strategies that protect against malaria infection.

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ZEB2$^+$ EFFECTOR CD4$^+$T CELLS CORRELATE WITH ANTIMALARIAL IMMUNITY BY RESTRAINING EFFECTOR CD8$^+$RESPONSES

Jennifer Enright[1], Yevel Flores Garcia[2], Fidel P. Zavala[2], Johanna Patricia Daily[1], Gregoire Lauvau[1]

[1]Albert Einstein College of Medicine, Bronx, NY, United States, [2]Johns Hopkins Univ Sch of Pub Hlth, Baltimore, MD, United States

Malaria is a major global health challenge, with over 200 million cases and 600,000 deaths annually. The two WHO-approved vaccines target the *Plasmodium falciparum (Pf)* circumsporozoite protein (CSP), expressed during liver stage infection. While anti-*Pf* antibodies correlate with naturally acquired clinical immunity, in which repeated exposure reduces fever and promotes asymptomatic infection, the cellular immune correlates of protection remain poorly defined. In a prospective cohort study in a malaria-

endemic region of Malawi, we previously identified a clonally expanded population of CSP-specific, cytolytic effector memory CD4+ T cells enriched among clinically immune individuals during infection. These cells express the transcription factor ZEB2, a regulator of terminal effector differentiation in CD8+ T cells, whose role in CD4+ T cells is poorly understood. We hypothesize that ZEB2+ effector memory CD4+ T cells are key contributors to protection against liver stage infection. Using radiation-attenuated sporozoite (RAS) immunization to induce robust liver-stage immunity in mice, we identified expanded ZEB2+ effector CD4+ T cells in blood and liver phenotypically comparable to those in the blood of malaria-infected humans. Upon infectious challenge, genetic depletion of ZEB2 in CD4+ T cells resulted in more robust CD4+ T cell expansion and IFNγ production compared to wildtype counterparts. Pathogen-specific CD8+ T cells also expanded more, expressed higher levels of activation markers, and produced more inflammatory cytokines. Consequently, ZEB2 depletion in CD4+ T cells resulted in increased mortality and worse cerebral function in the *P. berghei ANKA* model. Taken together, these results suggest that ZEB2 restrains CD4+ T cell differentiation, to limit terminal effector CD8+ T cell differentiation during *Plasmodium* infection. We therefore propose a model in which ZEB2+ effector memory CD4+ T cells contribute to clinical immunity not through direct effector functions, but by tempering excessive inflammation and immunopathology.

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FLUCTUATING HIGHER HUMIDITY DECOUPLES *ANOPHELES* IMMUNITY AND AMPLIFIES MALARIA TRANSMISSION POTENTIAL

Luna Dael, Yarno Valgaerts, **Maria Luisa Simoes**
Institute of Tropical Medicine Antwerp, Antwerp, Belgium

The malaria mosquito innate immune system is a major regulator of vector competence and malaria transmission. Despite mosquitoes being exposed to climate variations, the impact of realistic climatic fluctuations, particularly how daily humidity cycles affect *Anopheles* immune responses with repercussions for malaria transmission remains severely understudied. While a limited number of studies has focused on the role of temperature on mosquito immunity, standard laboratories still rely on static, optimized conditions that mask the physiological realities of vector-pathogen dynamics. To start addressing this issue, our laboratory pioneered a novel approach, exposing *Anopheles* mosquitoes from egg to adult to combined diurnal fluctuations in temperature and relative humidity, to investigate the impact of these realistic conditions on gene expression and fitness (Lemos-Silva *et al.* 2025). Building upon this novel approach, in this study we specifically investigated how fluctuating relative humidity (FH), simulating current (Low FH) conditions and potential future (High FH) climate change scenarios, alters *Anopheles stephensi* transcriptomic baselines and *Plasmodium falciparum* transmission. Our transcriptomic analyses using qRT-PCR revealed that High FH conditions force a severe, tissue-specific decoupling of the innate immune system. Exposure to future climate conditions actively suppressed the local midgut complement pathway while priming systemic detoxification and oxidative stress machinery. This environmentally driven physiological shift translated into extreme, divergent infection phenotypes, where High FH mosquitoes harbored significantly higher *Plasmodium falciparum* numbers compared to Low FH mosquitoes. Ultimately, our findings demonstrate that elevated humidity simulating future climate change drives *Anopheles* away from active resistance and towards immune tolerance. This physiological trade-off significantly amplifies vector competence, strongly suggesting that future climatic shifts related to increased humidity will exacerbate malaria transmission potential in the field.

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MALARIA PARASITE DEVELOPMENT AND VECTOR COMPETENCE ARE SHAPED BY HEME BUFFERING IN THE MOSQUITO MIDGUT

Francis De Souza Saraiva[1], Thiago Luiz Alves E Silva[1], Jose L. Ramirez[2], Joel Vega-Rodriguez[1]
[1]NIH, Rockville, MD, United States, [2]USDA, Peoria, IL, United States

Blood feeding dramatically reshapes the mosquito midgut by introducing a pulse of nutrients and iron-rich heme that alters the digestive environment. These conditions are key determinants of both microbiome structure and *Plasmodium* development. To test whether host-derived mechanisms for heme buffering could regulate these processes, we used a hemopexin-deficient (HPxKO) mouse model, which exhibits elevated circulating free heme compared to wild-type hosts. Using this model, we evaluated the effect of excess heme in the mosquito microbiome and mosquito tissue gene expression. We observed that in mosquitoes feeding on HPxKO mice, excess heme accelerated early microbial expansion, with a significant increase in total bacterial load at 24 hours post-feeding, coinciding with peak hemoglobin degradation. This initial expansion progressed into increased microbial richness and diversity by 48 hours compared to controls. Community restructuring was non-random: opportunistic taxa proliferated under elevated oxidative stress, whereas taxa typically associated with heme-rich environments, including *Serratia* and *Elizabethkingia*, were reduced under hemopexin-deficient conditions. Reflecting the altered environment under excess heme, midgut transcriptional responses at 72 hours post-feeding supported an imbalance in detoxification pathways and increased oxidative stress, together with delayed resolution of nutrient-sensing pathways and digestive processes. These changes were accompanied by a significant reduction in both early and late oocyst numbers, indicating impaired parasite establishment and development. Finally, by 96 hours post-feeding, following complete blood meal digestion, microbial communities converged, indicating that heme-driven diversification was transient and limited to the digestive window. Together, these findings show that control of heme exposure during blood meal digestion regulates midgut homeostasis, linking microbiome dynamics to *Plasmodium* development and ultimately influencing mosquito vector competence.

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MATING, JUVENILE HORMONE, AND MIDGUT REMODELING SHAPE IMMUNITY AND VECTOR COMPETENCE IN *AEDES AEGYPTI*

Alessandra Girard-Mejia[1], Norma Padilla[2], Mariana Wolfner[1], Laura C. Harrington[1], **Mabel Taracena Agarwal**[1]
[1]Cornell University, Ithaca, NY, United States, [2]Universidad Del Valle, Guatemala, Guatemala

Mosquito fecundity, longevity, and vectorial capacity are shaped by the physiological changes that follow mating, yet the post-mating changes in the mosquito midgut are poorly understood. In Aedes aegypti, we have demonstrated that mating triggers midgut growth through Juvenile Hormone (JH)-dependent signaling, with important downstream consequences for immunity, microbiota, and reproduction. JH suppresses midgut antimicrobial peptide (AMP) expression in a effector-specific manner, enabling expansion of the native bacterial community. This mating-induced shift — characterized by downregulation of specific AMPs and increased bacterial abundance — is associated with increased egg production and extended lifespan, revealing a JH-mediated cross-talk between gut epithelial remodeling, cell cycle regulation, and the trade-offs governing nutrition, immunity, and reproduction at the cellular level. Critically, we demonstrate that these physiological patterns are not limited to laboratory strains. In field-collected Ae. aegypti, mated females displayed significantly enlarged midguts relative to virgins, and gravid individuals showed reduced expression of some AMPs alongside increased bacterial abundance — mirroring laboratory findings. Laboratory-reared adults from field-collected immatures exhibited the same mating-dependent patterns, confirming the

robustness of this trade-off across ecological contexts. Importantly, mated females showed a trend toward higher Zika virus infection probability, suggesting that the reproductive physiology of the vector — and specifically the mating-induced immunosuppressive state of the midgut — may be an underappreciated determinant of arboviral transmission. Together, these findings position JH-regulated midgut remodeling as a central axis linking mosquito reproductive biology to vector competence, with potential implications for the design of more targeted and ecologically informed vector control strategies.

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DISRUPTION OF *ANOPHELES* ENOLASE BLOCKS MALARIA PARASITES THROUGH LEAKY GUT-INDUCED IMMUNE PRIMING

Yuemei Dong[1], Isaac Owusu-Frimpong[2], Shengzhang Dong[3], Mahnoor Abdullah[4], George Dimopoulos[5]

[1]Johns Hopkins University, Baltimore, MD, United States, [2]Johns Hopkins Sch of Public Health, Dept Molecular Microbiology & Immunology, Baltimore, MD, United States, [3]Baltimore, MD, United States, [4]Johns Hopkins School of Public Health MMI Dept, Baltimore, MD, United States, [5]Johns Hopkins Univ Sch of Public Health, Baltimore, MD, United States

Malaria remains a major global health challenge, and new vector control strategies are urgently needed. The mosquito midgut is a key barrier that influences *Plasmodium* survival during ookinete invasion. Previous studies showed that disruption of the prefoldin chaperonin complex impairs gut barrier function and produces a “leaky gut” phenotype, suggesting that structural components of the midgut are critical determinants of parasite survival. Among these factors, enolase, a major extracellular matrix-associated protein identified through co-immunoprecipitation with the prefoldin complex, emerged as a strong mosquito host factor for *Plasmodium* infection. RNAi-mediated silencing significantly reduced parasite loads in the mosquito midgut, and CRISPR/Cas9-mediated gene editing confirmed that enolase is essential for maintaining midgut integrity. Depletion of enolase after blood feeding caused gut distention and leakage of fluorescently labeled bacteria into the mosquito hemolymph, consistent with impaired barrier dysfunction. Using RNAi silencing, immunofluorescence confocal microscopy, qPCR profiling of antimicrobial and anti-*Plasmodium* genes in the presence and absence of microbiota, and conditional CRISPR/Cas9 knockout, we investigated the mechanism underlying enolase function as a *Plasmodium* host factor. Our findings suggest that enolase is essential for maintaining midgut barrier integrity, and that its inhibition disrupts epithelial and extracellular matrix structure, leading to a leaky gut that triggers microbe-mediated anti-*Plasmodium* immune priming and compromises parasite laminin-based immune evasion. Together, these results identify enolase as a key component of midgut integrity and parasite survival, highlighting a potential new target for malaria transmission-blocking interventions.

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PROGRAMMING THE *AEDES AEGYPTI* SIRNA PATHWAY TO DISRUPT ARBOVIRUS TRANSMISSION

Shengzhang Dong[1], Jessica Ciomperlik-Patton[2], Kevin M. Myles[2], George Dimopoulos[3]

[1]W. Harry Feinstone Department of Molecular Microbiology and Immunology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, United States, [2]Department of Entomology and AgriLife Research, Texas A&M University, College Station, TX, United States, [3]Johns Hopkins University Bloomberg School, Baltimore, MD, United States

Aedes aegypti transmits major arboviruses, including dengue (DENV), Zika (ZIKV), and chikungunya (CHIKV), which cause substantial global health and economic burdens. As insecticide resistance and climate change undermine traditional control methods, genetic strategies to enhance mosquito immunity offer a sustainable alternative. In *Ae. aegypti*, the small interfering RNA (siRNA) pathway is the primary antiviral defense, in which Dicer-2 (Dcr2) processes viral double-stranded RNA into siRNAs that guide Argonaute-2 (Ago2) to degrade viral RNA. To engineer mosquitoes with robust antiviral resistance, we generated transgenic lines overexpressing key siRNA components, including *Dcr2, Ago2, R2D2, Loqs,* and *Loq2*. These were expressed under tissue-specific promoters, including vitellogenin, apyrase, carboxypeptidase, G12, and chymotrypsin, to program the pathway across various tissues and developmental stages. We also combined transgenes through genetic crosses to test synergistic effects. Our results show that the siRNA pathway can be programmed, as evidenced by increased viral siRNA production and reduced viral titers. Antiviral efficacy was highly context dependent: individual genes conferred different levels of protection in the midgut, and Dcr2 produced distinct effects depending on the tissue targeted. Notably, simultaneous overexpression of multiple key genes in the midgut produced the strongest antiviral effect and effectively reduced transmission of multiple arboviruses, particularly DENV. These findings highlight the complexity of siRNA-mediated immunity and support multi-gene, tissue-specific programming as a potential strategy to interrupt arbovirus transmission.

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MRNA COCKTAIL-LNP VACCINATION ENABLE IDENTIFICATION OF NOVEL MALARIA TRANSMISSION BLOCKING ANTIGENS

Yu-Min Chuang[1], Garima Dwivedi[2], Brady Graham Dolan[3], John Veenstra[1], Zhichao Zhou[1], Mohamad-Gabriel Alameh[2], Drew Weissman[2], Erol Fikrig[4]

[1]Yale University, New Haven, CT, United States, [2]University of Pennsylvania, Philadelphia, PA, United States, [3]Hershey, PA, United States, [4]Yale Sch of Medicine, New Haven, CT, United States

Malaria is a major life-threatening infection. Even though there are WHO-approved vaccines that target *Plasmodium falciparum*, the transmission is still a global concern and other *Plasmodium* species also cause disease. After a mosquito takes a blood meal from an infected host, a limited number of *Plasmodium* establish infection inside mosquito midguts, which serves as the bottleneck and offers an unique target to eliminate *Plasmodium* transmission. Transmission blocking vaccines (TBV) targeting mosquito antigens can potentially block the transmission of different Plasmodium species, but identification of potent antigens is always a challenge. To identify novel and unrecognized mosquito antigens as TBVs, we used multiantigen mRNA vaccine strategy to discover the essential mosquito TBV antigens. We synthesized 4 multi-antigens mRNA-LNP vaccines, each contained around up to 15 mosquito antigen-specific mRNAs. By active immunization and then transmission-blocking studies in mice, one of the four cocktail mRNA-LNP vaccine significantly reduced oocyst numbers. We further divided those antigens into three groups, each have 3-4 antigen-specific mRNAs. Two of these three groups immunization reduced oocyst numbers. Using purified proteins and immunoblots, three antigens, AGAP000610, AGAP002378 and AGAP010257 were recognized by our cocktail mRNA-LNP vaccine. AGAP000610 has been shown to block Plasmodium transmission in previous studies. We further proved that immunization against either AGAP010257 or AGAP002378 significantly reduced oocyst numbers, which can serve as new TBV candidates. Our strategy and results demonstrate that a multiantigen mRNA-LNP vaccine platform can rapidly identify novel TBV antigens. This can significantly overcome the issue about new antigens identification and shorten the preclinical stage.

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EVALUATION OF SALIVARY PROTEINS AS POTENTIAL TARGETS FOR MALARIA TRANSMISSION-BLOCKING STRATEGIES

Renuka Elizabeth joseph[1], Patricia Alvarenga[2], Abhai K. Tripathi[3], Joel Vega-Rodriguez[4]

[1]National Institute of Allergy and Infectious Diseases, Rockville, MD, United States, [2]NIAID/NIH, Rockville, MD, United States, [3]Johns Hopkins Malaria Research Institute, Baltimore, MD, United States, [4]NIH, Rockville, MD, United States

Malaria transmission depends on the passage of *Plasmodium* parasites between humans and *Anopheles* mosquitoes, with parasite uptake by the mosquito representing a critical bottleneck. During blood feeding, mosquitoes inject salivary proteins into the skin that modulate host hemostasis and inflammation to enable efficient blood uptake. These proteins are also co-ingested and enter the mosquito midgut lumen, where early parasite development occurs. Despite this exposure, it remains unclear how mosquito salivary proteins shape the midgut environment and influence the establishment of *Plasmodium*. Five mosquito salivary proteins—saglin, AAPP, D7L2, D7r2, and A5R1—were selected based on their abundance in saliva and anti-hemostatic and immunomodulatory functions. Immunohistochemical analysis of the *Anopheles gambiae* blood bolus confirmed that all five proteins were ingested. In mosquitoes that fed directly on mice, the blood inside the mosquito midgut showed pronounced compartmentalization, with clotted blood cells and regions enriched in salivary proteins. By contrast, mosquitoes fed on artificial blood feeders, which lack coagulation, immune, and inflammatory factors, showed a more dispersed distribution throughout the midgut lumen. These results indicate that natural feeding generates a midgut microenvironment that could influence the development of *Plasmodium*, which may be overlooked in artificial feeder studies. We analyzed the impact of these proteins on parasite transmission using direct and passive immunization in *Plasmodium berghei*-infected mice. Antibodies against D7L2 reduced midgut oocyst formation by ~80%, whereas antibodies against saglin, AAPP, D7r2, and A5R1 had no effect. Interestingly, the reduction in oocyst numbers by D7L2 antibodies was observed in Th2-prone BALB/c mice but not in Th1-prone C57BL/6 mice, suggesting that the effect is mediated by a Th2-biased immune response. Together, these findings confirm D7L2 as a key factor in parasite transmission and highlight mosquito salivary proteins as promising targets for next-generation malaria transmission-blocking interventions.

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DISCOVERY OF MOSQUITO FACTORS REGULATING *PLASMODIUM FALCIPARUM* TRANSMISSION THROUGH HOST-PARASITE INTERACTION MAPPING

Vandana Prasad

Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

Malaria transmission requires precise molecular interactions between *Plasmodium falciparum* ookinetes and the mosquito midgut, yet these interactions remain poorly defined. Here, we used Receptor-Antigen Profiling (REAP) with an *Anopheles gambiae* exoproteome library to systematically identify mosquito proteins that interact with key ookinete stage specific parasite proteins, including PPLP3-5 and CTRP. This approach revealed multiple candidate host factors, including the neuropeptide pyrokinin and several previously uncharacterized proteins. RNAi-mediated gene silencing in female *An. gambiae* demonstrated that knockdown of selected genes significantly reduced oocyst intensity following *P. falciparum* and *P. berghei* infection ($p = 0.0001$). RNAi-mediated silencing of selected candidates in *An. gambiae* significantly reduced parasite infection intensity in both *P. falciparum* and *P. berghei*, demonstrating their functional role in facilitating parasite development. Moreover, ELISA-based assays confirm direct interactions between the identified mosquito ligands and their corresponding parasite proteins. These results uncover previously unknown mosquito ligands of parasite survival and highlight critical molecular interfaces that can be targeted to block transmission. **Impact:** This study provides a scalable framework for mapping host-parasite interactions and identifies novel intervention targets, advancing efforts toward next-generation transmission-blocking strategies for malaria control.

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DISSECTING PROPHENOLOXIDASES (PPOS) CONTRIBUTIONS TO MELANIZATION-BASED REFRACTORINESS IN *ANOPHELES*

Cindy M. Kimura[1], Sally Saab[2], Yuemei Dong[3], George Dimopoulos[4]

[1]Johns Hopkins, Baltimore, MD, United States, [2]Johns Hopkins University, Baltimore, MD, United States, [3]Johns Hopkins Univ, Baltimore, MD, United States, [4]Johns Hopkins Univ Sch of Public Health, Baltimore, MD, United States

Prophenoloxidases (PPOs) are central components of the mosquito innate immune system, mediating melanization, a defense response that encapsulates and eliminates invading pathogens, including malaria parasites. However, the contributions of individual PPOs remain poorly defined. Here, we investigated the nine PPO genes in *Anopheles gambiae* using integrated transcriptional and functional approaches. Expression profiling across developmental stages (embryo, larval instars, pupae, and adults) revealed stage-specific regulation, with PPO2, PPO4, PPO5, PPO6, and PPO9 expressed in adults (ANOVA, $p < 0.05$). PPO3 showed the strongest response to blood feeding, with ~130-fold upregulation at 48 hours, indicating a role in post-feeding immune activation. RNAi-mediated silencing combined with CTL4 knockdown demonstrated that PPO3, PPO5, and PPO6 significantly contribute to melanization, as their silencing reduced Plasmodium berghei melanization ($p < 0.0001$). Co-silencing of PPO3, PPO6, and PPO9 also decreased melanization ($p < 0.05$). Transgenic lines overexpressing PPO5 and PPO6, and PO5 and PO6, under carboxypeptidase (CP) and vitellogenin (Vg) promoters showed no melanization phenotype in *Plamodium falciparum* infections, but significantly reduced infection intensity after pre-blood feeding induction (Mann-Whitney, $p < 0.0001$). In *P. berghei*, preliminary data indicate increased melanization and reduced oocyst survival. These findings reveal functional specialization within the PPO family and identify PPO3 and PPO6 as key mediators of melanization-based refractoriness and promising targets for malaria control strategies.

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PERSISTENT CROSS-REACTIVE ANTIBODY TITERS DESPITE WANING HETEROLOGOUS NEUTRALIZATION IN A FIVE-YEAR FOLLOW-UP OF A CONTROLLED DENGUE VIRUS-1 HUMAN CHALLENGE MODEL

Esther I. Adekomi, Patrick I. Mpingabo, Stephen J. Thomas, Adam T. Waickman

State University of New York Upstate Medical University, Syracuse, NY, United States

The four serotypes of dengue virus (DENV1-4) cause significant morbidity and mortality throughout the tropical and subtropical regions. Following a primary infection with one of the 4 DENV serotypes, the immune system generates a repertoire of antibodies classified as type-specific (TS) or cross-reactive (CR). Epidemiological evidence shows that long-term immunity to the infecting serotype is generally long-lasting, while CR immunity is short-lived. The mechanistic basis for this divergence remains a critical question in DENV immunology. Specifically, if this waning immunity is driven by a rapid decline in antibody concentration or a decline in the functional quality of the CR antibodies. To address this question, we utilized longitudinal samples from a controlled DENV1 human challenge model. In this study, flavivirus-naïve volunteers were challenged with an attenuated strain of DENV1, and blood samples were collected at multiple time points. These volunteers were called back after five years, and additional blood samples were collected up to day 180 following recall. To understand the functional kinetics of the TS and CR antibodies, we analyzed the

neutralization potency to all 4 serotypes using a reporter virus particle-based neutralization assay. Then, we quantified the abundance of the CR antibodies using a blockade of binding assay with the monoclonal antibody, 4G2, that targets the conserved fusion-loop epitope. Our results revealed that although neutralization against DENV2-4 decreases to sub-neutralizing levels over five years, 4G2-like CR antibodies persist in the plasma at relatively high levels. This observation that CR antibodies persist even after they fail to neutralize the virus identifies a critical window of vulnerability where persistent, non-neutralizing antibodies remain at high levels, potentially facilitating antibody-dependent enhancement. Together, these results provide longitudinal evidence that the waning of cross-protective immunity is driven by a qualitative loss of antibody functions rather than a quantitative decline in titers and have implications for measuring vaccine efficacy.

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STRUCTURAL BASIS FOR DIFFERENTIAL NEUTRALIZATION OF CLINICAL ISOLATES OF DENGUE VIRUS SEROTYPES 1 AND 2 BY MONOCLONAL ANTIBODIES FROM THE SAME HUMAN PUBLIC CLONOTYPE

Vashi Negi[1], Tulika Singh[1], Thomas Klose[2], Gerald Vasquez Aleman[3], Tiffany Kim[4], Eunyoung Kim[4], Elias Michael Duarte[1], Nharae Lee[1], Baldeep Khare[2], Steven Wolinsky[4], Angel Balmaseda[3], Richard Kuhn[2], Eva Harris[1]

[1]Division of Infectious Diseases and Vaccinology, School of Public Health, University of California, Berkeley, Berkeley, CA, United States, [2]Purdue Institute of Inflammation, Immunology and Infectious Disease, Purdue University, West Lafayette, IN, United States, [3]Sustainable Sciences Institute, Managua, Nicaragua, [4]Feinberg School of Medicine, Northwestern University, Chicago, IL, United States

The four dengue virus serotypes (DENV1-4) are mosquito-borne flaviviruses that cause widespread disease. Understanding how antibodies recognize and neutralize different DENV serotypes is essential for informing therapeutic and vaccine development efforts. Here, we used single-particle cryo-electron microscopy (cryo-EM) to compare structures of 3 monoclonal antibodies (mAbs) from a single human public clonotype bound to DENV1 and DENV2. These mAbs (#13, #23, and #24) were identified by single-cell sequencing of antibody-secreting B cells from children with acute DENV2 infection and a prior DENV1 infection from the Pediatric Dengue Cohort Study in Nicaragua. These mAbs originate from the same B cell clonotype (V_H3-30/V_L3-21) and differ only modestly in sequence. All three bind both serotypes and potently neutralize DENV1 (NT_{50}: 19, 1, and 3 ng/mL, respectively) but exhibit varying neutralization potencies against DENV2 (NT_{50}: 34 ng/mL, 122 ng/mL, and non-neutralizing, respectively). We used Nicaraguan clinical isolates of DENV1 and DENV2 to generate Fab-bound complexes. Structures were resolved to 2.9-3.6 Å resolution for DENV2 and 4.2-6.5 Å for DENV1 complexes. Comparative structural analyses revealed that all three Fabs bind the envelope domains DI-DII within a dimer near the N67 and N153 glycans and occupy equivalent binding sites near the icosahedral twofold and threefold axes of symmetry in the DENV1 asymmetric unit. On DENV2, Fabs #13 and #23 that retain moderate neutralization potency bind the same sites as seen in DENV1 but show low occupancy near the fivefold axis, which is not observed in DENV1. In contrast, Fab #24, which does not neutralize DENV2, exhibits greatly reduced overall virion occupancy and binds primarily near the fivefold axis. These structural data demonstrate that genomically related mAbs from sequential DENV1-DENV2 infections may recognize multiple sites on the virion and toggle site preference across DENV serotypes, resulting in differential neutralization. Thus, structural differences in virion binding can provide insights into DENV serotype-specific antibody recognition and neutralization.

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HUMORAL IMMUNE RESPONSES ASSOCIATED WITH PROTECTION AND DISEASE SEVERITY IN LASSA FEVER

Kerri Guth Lal[1], Zahra F. Parker[2], Melanie McCauley[3], Karina Ivette Rivas[4], Michelle Zemil-McCrea[1], Paul V. Thomas[1], Wei-Hung Chen[1], Sydney Mika[1], Abdulwasiu Bolaji Tiamiyu[5], Edward A. Akinwale[6], Iguosadolo Nosamiefan[7], Johnson C. Okolie[7], Bonnie M. Slike[8], Lily Metzger[1], Jaime Jensen[1], Agnes Hajduczki[1], Jeffrey Robert Currier[9], Samantha Townsley[1], Leigh Anne Eller[1], Nelson Michael[10], Sandyha Vasan[1], Aaron Farmer[11], Dominic Paquin-Proulx[1], Anise N. Happi[7], M. Gordon Joyce[1], Christian Happi[12], Shelly Krebs[13]

[1]Henry M. Jackson Foundation, Silver Spring, MD, United States, [2]The Henry M. Jackson Foundation for the Advancement of Military Medicine (HJF), Ikoyi, Nigeria, [3]Henry Jackson Foundation, Bethesda, MD, United States, [4]Laurel, MD, United States, [5]Walter Reed Program Nigeria, Abuja, Nigeria, [6]HJF Medical Research International (HJFMRI) Ltd Gte, Abuja, Nigeria, [7]Institute of Genomics and Global Health (IGH), Ede, Nigeria, [8]Henry M Jackson Foundation, Rockville, MD, United States, [9]WRAIR Walter Reed Army Inst of Research, Silver Spring, MD, United States, [10]WRAIR, Silver Spring, MD, United States, [11]AFRIMS, Silver Spring, MD, United States, [12]ACEGID, REDEEMER'S UNIVERSITY, NIGERIA, Ede, OS, Nigeria, [13]Walter Reed Army Institute of Research, Silver Spring, MD, United States

Lassa virus (LASV), the causative agent of Lassa fever (LF), infects approximately 400,000 individuals annually in West Africa through exposure to infected rodent excreta. While ~80% of infections result in mild disease, severe LF is characterized by hemorrhagic fever and multiorgan dysfunction, with a fatality rate of ~1%. Despite this burden and the absence of FDAapproved vaccines or therapeutics, immune correlates of protection against LF remain poorly defined. To address this, we analyzed convalescent plasma antibody binding, neutralization, and Fcmediated effector functions at 6 and 12 months post infection in LASV infected adults from endemic southern Nigeria (EID032). 20 individuals developed mild (asymptomatic) LF, and 20 hospitalized participants developed severe LF. Individuals with mild LF, wherein preinfection samples were available, exhibited preexisting LASV binding, neutralizing, and Fceffector antibody responses, consistent with prior exposure, with no increase in response magnitude following infection. In contrast, survivors of severe LF had high and sustained levels of LASV IgG binding, neutralization, antibodydependent complement deposition (ADCD), and opsonization in convalescence. Compared with mild LF, severe LF developed higher antibody binding, neutralization, ADCD, and opsonization at both in convalescence. Severe LF survivors also demonstrated broader antibody binding across LASV glycoprotein complex (GPC) lineages I–IV and across 13 additional Old World and New World arenaviruses, indicating robust crossreactive humoral responses. Neutralization potency increased between 6 and 12 months postinfection, reaching ID80 titers approximately three orders of magnitude above negative controls, and correlated with arenavirus crossbinding, suggesting targeting of conserved neutralizing epitopes. Together, these findings suggest that mild LF may be associated with preexisting protective humoral immunity, whereas severe LF elicits durable, high magnitude, and broadly crossreactive antibody responses, informing immune correlates of protection and LASV vaccine development.

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COMPARISON OF B CELL REPERTOIRE ELICITED BY TWO ZIKA VIRUS VACCINE PLATFORMS IN FLAVIVIRUS-NAIVE PARTICIPANTS

Vincent Dussupt[1], Helene Fradin-Kirshner[2], Ninaad Lasrado[3], Samantha M. Townsley[1], Michael K. McCracken[4], Bonnie M. Slike[1], Samantha Balgobin[2], Letzibeth Mendez-Rivera[1], Lily Metzger[1], Lauren Smith[1], Caitlin Kuklis[4], Gabriel Smith[1], Philip K. Ehrenberg[4], Aviva Geretz[1], Jaime L. Jensen[1], Paul V. Thomas[1], Giomar Burgos-Rosario[1], James D. Brien[5], Nelson L. Michael[4], Aaron R. Farmer[4], Gregory D. Gromowski[4], Rasmi Thomas[4], M. Gordon Joyce[1], Kevin Wiehe[2], Dan H. Barouch[3], Shelly Krebs[4]

[1]Walter Reed Army Institute of Research, Henry M. Jackson Foundation for the Advancement of Military Medicine, Silver Spring, MD, United States, [2]Human Vaccine Institute, Duke University, Durham, NC, United States, [3]Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, United States, [4]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [5]University of Kentucky, Lexington, KY, United States

Following the 2015-16 Zika virus (ZIKV) epidemic, a Zika purified inactivated vaccine (ZPIV) and an Ad26.ZIKV.M.E. vaccine (Ad26) were tested in phase 1 trials. Both platforms elicited similar binding and neutralizing antibody responses. To investigate the B cell repertoire, we sorted ZIKV-reactive B cells, using a combination of whole virus and envelope (E) protein probes, from 10 participants from each study and obtained over 3,300 paired heavy and light chain B cell receptor (BCR) sequences. While general antibody repertoire features were similar for both vaccines, we observed significant enrichment of VH3-23, VH4-31, VH5-10, VH3-64D and VH3-20 heavy chain genes in sequences from the ZPIV study and VH5-10 for the Ad26 group. Prevalent BCR clonotypes as well as those from clonally expanded families and singletons with high reactivity to ZIKV E were expressed as IgG monoclonal antibodies (mAbs) (N=180). MAbs were tested for epitope mapping in E, cross-reactivity and neutralization. In the ZPIV group (N=82), 56% of the mAbs were ZIKV-specific, with half targeting DII, followed by DI, DIII and the remainder distributed between E quaternary-like and DI/III interface. MAbs that cross-reacted to other flaviviruses mainly targeted DII. In contrast, in the Ad26 group (N=98), we observed a larger fraction (83%) of ZIKV-specific mAbs, targeting DII and quaternary-like E epitopes. Neutralizing antibodies were almost exclusively ZIKV-specific (72% and 93% for the ZPIV and Ad26 groups, respectively), where ZPIV-elicited neutralizing mAbs targeted DII, DIII and DI and their diverse heavy chain gene usage was dominated by VH3-23 and VH3-30. In contrast, Ad26-elicited neutralizing mAbs targeted DII and quaternary-like E epitopes and preferentially used VH5-10. Representative mAbs from both groups protected mice from ZIKV replication at a low 10 µg (0.5 mg/kg) dose. Overall, the two ZIKV vaccine platforms elicited different antibody specificities with ZPIV offering a broader response against multiple E epitopes while Ad26 led to a more focused response against DII. These data may have important implications for future ZIKV vaccine development.

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DOMINANT FUSION LOOP RECOGNITION BY THE MEMORY B CELL REPERTOIRE AFTER MULTIPLE DENGUE VIRUS INFECTIONS

Kelsey E. Lowman[1], Rosemary A. Aogo[1], Sarah Smith[2], Saba Firdous[1], Ysabelle Broderson[1], Gitanjali Bhushan[1], Devina Thiono[1], Patrick I. Mpingabo[1], Amy R. Henry[2], Timothy Johnston[2], Jesmine Roberts-Torres[2], Luis Estrada[2], Chaim Schramm[2], Krista Gangler[3], Jeffrey I. Cohen[3], Sonya Jacobsen[4], Edgar Davidson[4], Barbara Blacklaws[5], Jonathan L. Heeney[5], Aravinda de Silva[6], Daniel C. Douek[2], Leah C. Katzelnick[1]

[1]Viral Epidemiology and Immunology Unit, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States, [2]Human Immunology Section, Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States, [3]Medical Virology Section, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States, [4]Integral Molecular, Philadelphia, PA, United States, [5]Department of Veterinary Medicine, University of Cambridge, Cambridge, United Kingdom, [6]School of Medicine, University of North Carolina, Chapel Hill, NC, United States

Antibodies play both a protective and enhancing role in dengue virus (DENV) infection. However, the composition of the cross-reactive memory repertoire and how much of it contributes to protection following secondary infection remains uncertain. As such, we characterized the repertoire of a highly DENV-exposed individual with broad flavivirus immunity using the "rapid assembly, transfection, and production of immunoglobulins" pipeline. Using stabilized envelope (E) dimer proteins as antigen-specific B cell probes, we identified 227 DENV-binding antibodies. Nearly 70% of the binding repertoire lost dimer binding when the E fusion loop (FL) was mutated (FL-sensitive antibodies), highlighting FL immunodominance within the memory B cell repertoire. From the B cell receptor sequences, we found *IGHV3* and *IGHV4* germline use and more negatively charged and less aromatic CDRH3s were predictive of antibody FL sensitivity. Although 12 of our most neutralizing antibodies demonstrating broad cross-serotype binding, those that bound E dimer with and without a mutated FL (FL-agnostic) showed potent DENV neutralization but with a restricted neutralization breadth. None of the four most potently neutralizing, FL-agnostic antibodies mapped to known quaternary epitopes like the broadly neutralizing E dimer epitope (EDE). Three mapped to the A-strand in E domain III and one mapped to the bc-loop in E domain II, targets previously identified in the repertoire after secondary infection. Conversely, the top neutralizing FL-sensitive antibodies demonstrated broader but weaker neutralization, even cross-neutralizing Zika virus and a West Nile chimera. Unlike for the whole FL-sensitive repertoire, this neutralizing subset contained CDRH3 properties more like FL-agnostic antibodies, including a greater aromatic residue content equal to that observed in known EDE antibodies. Consequently, we propose the FL epitope is important in the development of broadly neutralizing antibodies, and one mechanism in which the repertoire evolves after secondary infection is through shaping FL antibodies into greater contributors toward protection.

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A COMPARATIVE ANALYSIS OF THE IMMUNOTRANSCRIPTOMIC FEATURES OF DENV-1, -3 AND -4 HUMAN CHALLENGE MODELS

Celine S. C. Hardy[1], Lisa Ware[2], Heather Friberg[3], Joel V. Chua[4], Kirsten E. Lyke[5], Stephen J. Thomas[6], Adam Waickman[1]

[1]SUNY Upstate Medical University, Syracuse, NY, United States, [2]SUNY Upstate Medical Univ, Syracuse, NY, United States, [3]Walter Reed Army Inst of Res, Silver Spring, MD, United States, [4]Institute of Human Virology, Univ. of Maryland, Baltimore, MD, United States, [5]Univ of Maryland School of Medicine, Baltimore, MD, United States, [6]Skaneateles, NY, United States

Dengue virus (DENV) infection causes a range of clinical symptoms, from mild febrile illness to severe disease. Pre-existing DENV immunity contributes to this heterogeneity; however, substantial variability in clinical symptoms exists even among flavivirus-naïve individuals. In Dengue Human Infection Models (DHIM), participants are challenged with underattenuated DENV strains, providing an invaluable tool for studying the early virologic, transcriptional, and immunologic features of infection. DHIM studies using DENV-1, DENV-3, and DENV-4 have demonstrated qualitatively distinct clinical presentations across models. In both DHIM and natural infection, higher levels of DENV RNAemia are associated with symptom severity. However, the contribution of RNAemia levels and serotype to divergent transcriptional and clinical profiles in these challenge models remains unclear. In this work, we perform a comparative analysis of DHIM-1, DHIM-3, and DHIM-4 challenge studies to determine shared and unique features of the transcriptional response to infection and their associations with RNAemia and clinical symptoms. Differential gene expression analysis comparing day of peak viremia to baseline identified a conserved set of 290 upregulated genes across challenge models, indicating a core antiviral response regardless of serotype. Contrastingly, DHIM-3 challenge subjects demonstrated unique upregulated (n= 349) and downregulated

(n=266) genes, where pathway analysis indicated decreased cytoplasmic translation. These unique signatures were driven by DHIM-3 subjects with elevated RNAemia and severe symptomatology. To determine whether these transcriptional signatures represent serotype-specific effects or differences in viral burden, primary human PBMCs were exposed *in vitro* to DENV-1 or -3 at varying titers. Bulk RNA sequencing demonstrated that conserved and unique gene expression signatures varied as a function of viral dose rather than serotype. These data suggest that viral burden drives transcriptional responses following DENV infection, contributing to our understanding of dengue pathogenesis and immunity.

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DENGUE-SPECIFIC T CELL IMMUNITY INFLUENCED BY VARIOUS LEVELS OF PRE-EXISTING IMMUNITY

Amparo Martinez-Perez[1], Rosemary Aogo[2], Ysabelle Broderson[3], Kelsey Lowman[3], Camila D. Odio[4], Leah Katzelnick[5], **Daniela Weiskopf**[6]

[1]La Jolla Institute for Immunology, La Jolla, CA, United States, [2]NIAID, National Institutes of Health, United States, [3]NIAID, National Institutes of Health, Bethesda, MD, United States, [4]NIAID, Silver Spring, MD, United States, [5]NIAID/NIH, Bethesda, MD, United States, [6]La Jolla Inst for Immunology, La Jolla, CA, United States

Dengue virus (DENV) is a mosquito-borne flavivirus responsible for substantial global morbidity and mortality, with an estimated 300 million infections annually. Prior exposure to different DENV serotypes shapes subsequent immune responses, influencing both protective immunity and the risk of severe disease. This complexity poses a major challenge for the development of a universally effective and safe dengue vaccine.T cells represent a critical component of dengue immunity, functioning independently of antibody-mediated responses. In this study, we evaluated dengue-specific T-cell responses elicited by a live-attenuated monovalent DENV3 vaccine candidate, strain rDEN3Δ30/31-7164. Participants with varying levels of pre-existing DENV exposure were included, and viral challenge resulted in different levels of viremia among individuals. This model enabled us to closely track immune responses specific to the DENV-3 serotype providing a unique view of cellular immune dynamics. DENV-specific T-cell responses were characterized using multiparametric flow cytometry at baseline and at 15, 28, and 90 days following vaccination. The challenge induced measurable antigen-specific T-cell responses targeting both structural and non-structural viral proteins. We observed substantial inter-individual variability in T-cell responses, influenced by baseline serostatus and viremia levels. Notably, we detected a high proportion of cytotoxic $CD4^+$ and $CD8^+$ T cells, and as well as differences in the magnitude of T cells recognizing structural versus non-structural viral epitopes.Our study provides a detailed model of T-cell-mediated immunity following an attenuated DENV-3 challenge. These results provide new insight into DENV-specific immunity with relevance for future vaccine design.

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ALPHAVIRAL INFECTION IS CHARACTERIZED BY CXCL10-DRIVEN CD4+ T CELL RESPONSES AND LIMITED CD8+ T CELL MEDIATED ANTIVIRAL IMMUNITY

Sainetra Sridhar, Bobby Brooke Herrera

Rutgers, New Brunswick, NJ, United States

O'nyong-nyong virus (ONNV) is a mosquito-transmitted alphavirus closely related to chikungunya virus (CHIKV) that causes fever and debilitating polyarthritis in humans, with disease severity and resolution influenced by host age. The immunological mechanisms governing persistent inflammation and ineffective viral clearance remain incompletely defined. Using a murine model of ONNV infection spanning weanling (3 weeks), young adult (6-8 weeks), and aged mice (>6 months), we identify CXCL10 as a key regulatory of T cell composition and function at sites of infection. ONNV infection induced sustained *cxcl10* expression and age-associated viral persistence in infected tissues, accompanied by preferential accumulation of CD4+ T cells and limited recruitment of CD8+ T cells. In the draining popliteal lymph node, CD4+ but not CD8+ T cells upregulated CXCR3 and activation markers, consistent with selective chemotactic responsiveness to CXCL10. Moreover, both CD4+ and CD8+ T cells expressed elevated levels of T-bet and Roryt transcription factors, suggesting biased transcriptional programming governing effector differentiation. Perturbation of this axis using a CXCL10-neutralizing antibody or pharmacologic downregulation via atorvastatin reduced CD4+ T cell accumulation, altered T cell differentiation states, and decreased tissue viral burden, concomitant with reduced inflammation. Separately, we found that ONNV infection in vitro and in vivo may impact antigen presentation by downregulating MHC-I expression on infected cells, thus limiting CD8+ T cell priming. Taken together, the reduced antigen presentation and elevated CXCL10 biases the programming and recruitment of T cells to the site of infection. The resulting immune response is highly proinflammatory but poorly effective in mediating viral clearance, thus setting the stage for chronic disease.

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IMMUNE CHECKPOINT-MEDIATED T CELL DYSFUNCTION DRIVES AGE-DEPENDENT SUSCEPTIBILITY TO LA CROSSE VIRUS ENCEPHALITIS

Reem Alatrash[1], Bobby Brooke Herrera[2]

[1]Rutgers, The State University of New Jersey, New Brunswick, NJ, United States, [2]New Brunswick, NJ, United States

La Crosse virus (LACV) causes severe neuroinvasive disease predominantly in children, yet the immunological mechanisms underlying this age-dependent susceptibility remain poorly defined. Here, we investigated antiviral T cell responses during acute LACV infection using a murine model of pediatric disease. Infection in weanling mice, but not adults, was characterized by early and sustained engagement of inhibitory immune pathways. Weanling mice exhibited marked upregulation of the immune checkpoints programmed cell death protein 1 (PD-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) on $CD4^+$ and $CD8^+$ T cells, increased PD-L1 expression in lymphoid and central nervous system tissues, impaired effector T cell differentiation, and accumulation of atypical, poorly differentiated T cell populations. These defects were accompanied by reduced IL-2/STAT5 signaling and expansion of a $PD\text{-}1^{hi}$ regulatory T cell compartment. Functional targeting of checkpoint pathways revealed their role in constraining antiviral immunity. PD-1 blockade restored effector $CD8^+$ T cell differentiation and enhanced antigen-specific cytotoxic responses. Similarly, CTLA-4 blockade increased $CD8^+$ T cell activation and virus-specific granzyme B responses following stimulation with LACV antigens, accompanied by reduced viral burden in the brain. Dual blockade of PD-1 and CTLA-4 further reduced viral load, supporting a cooperative role for inhibitory checkpoint signaling in limiting effective antiviral T cell responses in young hosts. Together, these findings identify immune checkpoint signaling as a central mechanism underlying impaired antiviral T cell immunity during LACV infection and provide a mechanistic framework for age-dependent susceptibility to severe disease.

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DIGITALIZATION OF ROUTINE SEASONAL MALARIA CHEMOPREVENTION (SMC) DATA COLLECTION IN MALI FOR IMPROVED EFFICIENCIES

Boubacar Sidiki Maiga[1], Cheikh Gassama[1], KEITA Fatoumata[1], Alassane BANGOURA[1], Suzanne Van Hulle[2], Yameni Chrestien[3], Mady Cissoko[4], Aissata Kone[4]

[1]Catholic Relief Services - CRS, Bamako, Mali, [2]Catholic Relief Services, Baltimore, MD, United States, [3]Catholic Relief Services, Dakar, Senegal, [4]National Malaria Control Program - Mali, Bamako, Mali

Seasonal Malaria Chemoprevention (SMC) is a key intervention for reducing malaria-related morbidity and mortality among children aged 3 to 59 months in Mali. Following the introduction of digitalization of campaigns in 2022, an innovative approach to routine implementation, incorporating a digital system for individual data collection and monitoring, was piloted in 2025 to improve efficiency and sustainability. The intervention was

implemented in two health zones (Benguéne and N'Golokouna) in the Bla district, using a DHIS2 Tracker system that enabled individual enrollment and monitoring of children. Community health workers and health relays used smartphones for registration and administration of the SPAQ. Each child was uniquely identified using a SMC card with a QR code, ensuring traceability and minimizing duplicate entries. Vaccinations were administered at fixed sites (health facilities and community health posts). In total, 26 data elements and 39 indicators were configured for monitoring, disaggregated by sex and age. The approach demonstrated strong operational results, with 81% coverage among targeted children. The availability of real-time data significantly improved oversight and responsiveness, particularly for identifying low-performing areas. Digitalization has strengthened individual traceability and reduced errors associated with paper-based tools. The routine model, relying on community health workers integrated into the health system, has led to a 50-60% reduction in operational costs compared to the campaign-based approach. Challenges remain, including connectivity in rural areas, proficiency with digital tools, and seasonal constraints related to agricultural activities. The routine digitalization of SMC in Mali is feasible, efficient, and improves data quality and operational performance. This approach offers a promising and replicable model for nationwide implementation and for the sustainable strengthening of health information systems in resource-limited settings.

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UNDERSTANDING IMMUNIZATION GAPS IN PAKISTAN A HEALTH SYSTEMS AND BEHAVIORAL ANALYSIS USING CLIENT EXIT INTERVIEWS

Ahmad Khan[1], Imtiaz Hussain[1], Muhammad Umer[1], Shabina Ariff[2], Sajid Bashir Soofi[3]

[1]Aga Khan University, Karachi, Pakistan, [2]Aga Khan University, Karachi 74800, Pakistan, [3]Aga Khan University, Pakistan, Karachi, SD, Pakistan

Routine immunization coverage in Pakistan remains suboptimal, with persistent inequities across regions and population groups. Evidence on caregiver experiences at service delivery points is limited but critical for understanding barriers to vaccine uptake and completion. A cross-sectional Client Exit Interview (CEI) survey was conducted among caregivers of children under two years across 31 randomly selected districts. Districts were categorized by Penta3 coverage (low, medium, high), followed by stratified random selection of health facilities based on vaccination volume (2024). Using a three-stage cluster sampling design, 3,166 interviews were conducted at 279 facilities. Analysis was guided by the Journey to Health and Immunization framework: knowledge, intent, access, service delivery, experience of care, and follow-up. Descriptive analysis assessed barriers, and missed opportunities for vaccination (MOVs) were evaluated using records. Barriers were identified across all domains. Knowledge gaps included difficulty distinguishing routine from campaign vaccination (83.6%) and poor understanding of vaccination cards (61.8%). Confidence issues included vaccine hesitancy (32.5%), fear (31.5%), and safety concerns (30.0%), with 44.4% exposed to misinformation. Gender norms limited intent, with over 60% of mothers requiring permission to seek vaccination. Access barriers were substantial: 64.1% reported difficulty accessing services, 56.4% cited transport issues, and over 60% faced financial constraints. Facility gaps included failure to assess vaccination status (74.6%) and lack of referral (90.3%). Poor communication affected 40.4% of caregivers. Post-service gaps included lack of counseling (25.3%) and inadequate follow-up (41.6%). Overall, 23.1% of children experienced MOVs. In conclusion, Despite high service contact, both demand- and supply-side barriers impede completion of vaccination. Strengthening service delivery and addressing structural and sociocultural constraints are essential to reduce missed opportunities and improve equitable coverage.

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REACHING ZERO-DOSE AND UNDERVACCINATED CHILDREN IN TOGO: WHEN SEASONAL MALARIA CHEMOPREVENTION (SMC) CAMPAIGNS BECOME AN ENTRY POINT TO VACCINATION

Kwami G. Azialey[1], Yassimine A. Abou[1], Narcisse Makou[1], Patrick Palanga[1], Kessy A. Sekpe[1], Léonardo Shamamba Kinyungu[1], Tracy Kapezi[1], Somiablo Atekpe[2], Amevegbé K. Boko[3]

[1]Catholic Relief Services, Lomé, Togo, [2]National Malaria Control Program - Togo, Lomé, Togo, [3]Immunization Division (DI) / Expanded Program on Immunization (EPI), Lomé, Togo

Zero-dose and under-vaccinated children remain a major concern and pose a significant risk for outbreaks. In Togo, from 2019 to 2022, the proportion of zero-dose children ranged from 10% to 13% according to UNICEF. SMC campaigns in Togo are conducted using a doorto-door strategy, providing an opportunity for community health workers (CHW) to verify vaccination status of EPI targeted children. A cross-sectional study was carried out from June 1 to November 30, 2025, in 4 health districts of the Plateaux region during 5 cycles of the SMC campaign to estimate identification rates, completeness of referrals, and community acceptability. Preparatory activities included coordination meetings between NMCP, EPI and partners; updating tools and training modules; and training actors at all levels. Data collection through a digital (Lafia) application installed on CHWs' smartphones. A total of 2,392 under-vaccinated children and 2,312 zero-dose children were identified in households. Nearly 90% of these children lived in areas with limited access to healthcare services. All identified children were referred to the nearest health facility using a referral form. To ensure completion of referrals, an e-tracker system was set up from one cycle to the next to update the vaccination status of each child in the Lafia application. Overall, 2,186 (91.39%) under-vaccinated children and 2,145 (92.77%) zero-dose children were successfully caught up and linked to vaccination services. Missing vaccines among under-vaccinated children primarily included the combined measles-rubella vaccine, the second dose of inactivated polio vaccine, the yellow fever vaccine, and the meningitis vaccine. Integrating the identification of zero-dose and under-vaccinated children into SMC campaigns represents a promising approach to improving vaccination equity.

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A SPIRULINA-BASED PLATFORM FOR SCALABLE, THERMOSTABLE, NEEDLE-FREE VACCINES

Wadzanai P. Mboko[1], Melanie Janet Shears[2], Troy Paddock[1], Annette M. Seilie[3], Kanchan Aggarwal[1], Alex Pollock[1], David Bollick[1], Madison Kahwaty[1], Sebastian A. Mikolajczak[1], Sean C. Murphy[4], Nhi Khuong[1], Jim Roberts[1]

[1]Lumen Bioscience, Seattle, WA, United States, [2]University of Washington, Seattle, WA, United States, [3]University of Washingon, Seattle, WA, United States, [4]Univ of Washington, Seattle, WA, United States

Needle-free vaccines that combine robust immunity with simple manufacturing, thermostability, and practical delivery could substantially expand global access to immunization. However, many current vaccine platforms remain constrained by costly, complex manufacturing, dependence on purified antigens and added adjuvants, invasive administration, and strict cold-chain requirements that limit deployment in resource-constrained settings. These challenges are especially acute for structurally complex immunogens such as virus-like particles (VLPs), which can be difficult to manufacture at scale in stable formulations suitable for mucosal delivery. Here, we present a mucosal vaccine platform based on the edible photosynthetic cyanobacterium *Arthrospira/Limnospira platensis* (spirulina), which enables low-cost, scalable production of complex antigens directly in biomass, avoids extensive downstream purification, and supports storage without refrigeration. Using a *Plasmodium falciparum* NANP antigen displayed on woodchuck hepatitis virus-derived VLPs, we previously showed that spirulina-based intranasal boosters elicited durable antigen-specific systemic IgG responses in mice that persisted for up to

one year. We further show that spirulina lysates can be spray-dried and rehydrated while preserving VLP assembly, with stability maintained for at least four months at room temperature, eliminating the need for cold-chain storage. Intranasal administration provides a noninvasive route to systemic immunity, and immunogenicity is achieved without exogenous adjuvants. In nonhuman primates, repeated mucosal dosing was well tolerated, with only mild, transient effects observed. Together, these findings support spirulina as a versatile vaccine platform that integrates manufacturability, thermostability, and needle-free delivery, with potential to improve vaccine access across diverse antigens and infectious diseases.

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CONTROLLED TEMPERATURE CHAIN (CTC) VERSUS STANDARD COLD CHAIN (SCC) FOR ORAL CHOLERA VACCINE (OCV) DELIVERY: PROGRAMMATIC EFFICIENCY FROM A LARGE-SCALE VACCINATION CAMPAIGN IN ZAMBIA

Guissimon Phiri[1], **Shiferaw Tesfaye Tilahun**[2], Yubin Lee[2], Yeonji Jeon[2], Saemna Park[2], Sydney Chisala[3], Kasoma Puta[3], Dae-Hyup Koh[2], NamHoon Yeon[2], Peter J. Chipimo[4], Fred Kapaya[5], Ju Yeon Park[2], Deok Ryun Kim[2], Masuzyo Zyambo[6], Michelo Choongo[7], Marion Menozzi[8], Jean-Bernard Le Gargasson[8], Catherine Makwe[9], Vincent Mendiboure[10], Tarun Saluja[2], Jessica Cowden[2], Jerome H. Kim[2], John D. Clemens[2], Julia A. Lynch[2], Anna-Lea Kahn[11], Clement Lugala[4], Jacob Sakala[1], Isabel Meleki[1], Kennedy Lishimpi[1], Roma Chilengi[6], Se Eun Park[2]

[1]Ministry of Health, Lusaka, Zambia, [2]International Vaccine Institute, Seoul, Republic of Korea, [3]Chienge Distric Health Office, Chienge, Zambia, [4]World Health Organization, Lusaka, Zambia, [5]World Health Organization, Nairobi, Kenya, [6]Zambia National Public Health Institute, Lusaka, Zambia, [7]Zambia Statistics Agency, Lusaka, Zambia, [8]Gavi, the Vaccine Allience, Geneva, Switzerland, [9]International Federation of Red Cross and Red Crescent Societies, Geneva, Switzerland, [10]World Health Organization, Geneva, Switzerland, [11]Swiss Tropical and Public Health Institute, Basel, Switzerland

Controlled temperature chain (CTC) is an innovative approach that allows vaccines to be kept outside the standard cold chain (SCC) for a limited period under monitored conditions. CTC for oral cholera vaccine (OCV) delivery could improve vaccination coverage and reduce operational burdens associated with SCC. However, evidence on the feasibility and programmatic efficiency of CTC-for-OCV remains limited. A two-dose Euvichol-Plus preventive OCV campaign was conducted in 2025 in Chienge District, Luapula Province, one of the Priority Areas of Multisectoral Interventions (PAMIs) for cholera elimination in Zambia. Six study areas were pair-matched and randomly assigned to either CTC or SCC arm (1:1 ratio). 50 vaccination teams (25 in each arm) participated in vaccination of approximately 100,000 individuals aged ≥1 year. A trained study enumerator was attached to each vaccination team for study specific data collection using daily vaccination summary, temperature monitoring (vaccine vial monitor and peak temperature threshold indicator), and time motion log sheets. 99,917 and 99,950 doses were administered in 1st and 2nd rounds, respectively. Mann-Whitney U test (in R version 4.5.2) was used to compare parameters of programmatic efficiency of CTC-versus-SCC approach in the two arms. The CTC arm demonstrated significantly greater operational efficiency, with a shorter mean daily campaign duration (by 75 minutes; $p<0.001$). Although the increase in daily doses administered in CTC arm (28 additional doses on average) did not reach conventional levels of statistical significance ($p=0.073$), the vaccination rate was significantly higher, with 14 more individuals vaccinated per hour ($p<0.001$). Daily campaign duration in CTC arm exhibited 70- and 80-minutes shorter duration on average in 1st and 2nd round, respectively. These findings support the operational advantage of CTC delivery for OCV in similar resource-limited and hard-to-reach settings. Further assessments on CTC cost-efficiency and scalability are recommended in the future to inform broader implementation and policy engagement.

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CHARACTERIZING INJURY MORTALITY IN TWO RURAL REGIONS OF KENYA: RESULTS FROM A VERBAL AUTOPSY PROGRAM

Sophia Fabrizio[1], Peter S. Larson[2], Satoshi Kaneko[3], Violet Wanjua[4], Elora Shakoor[1]

[1]University of Michigan, Ann Arbor, MI, United States, [2]University of Michigan, Manchester, MI, United States, [3]Nagasaki Univ Nairobi Research Station, Nagasaki, Japan, [4]Kenya Medical Research Institute, Nairobi, Kenya

Injuries account for approximately 4.4 million deaths annually, with a disproportionate burden in low- and middle-income countries. In rural settings, injury risk is shaped by socioeconomic and environmental factors, yet population-based evidence linking injury mortality to these contexts remains limited in sub-Saharan Africa. We analyzed verbal autopsy data from a Health and Demographic Surveillance System operating across two rural Kenyan regions: Mbita along Lake Victoria, and Kwale County on the southern coast. Causes of death were assigned using standardized verbal autopsy procedures. Injury deaths were characterized by mechanism, age, sex, region, and season. Household socioeconomic indicators, geographic access to services, and environmental variables were linked to mortality records for descriptive and regression analyses. Among 208 injury-related deaths identified, violence (19.7%) and road traffic injuries (17.8%) were the leading causes, followed by drowning (10.6%), falls (10.0%), and poisoning or overdose (9.6%). Injury mortality peaked among those aged 13-29, though young children also experienced substantial mortality. Males comprised approximately 68% of deaths. Regional differences were pronounced: Mbita had higher proportions of drowning, suicide, and burns, while Kwale exhibited higher proportions of falls and snakebite deaths. Drowning deaths were significantly elevated during the wet season and drought periods. Only 31.7% of injury-related deaths occurred at a health facility, underscoring systematic under-counting in hospital-based surveillance. Injury deaths were more common among lower socioeconomic households. Injury mortality in these rural Kenyan regions is substantial and heterogeneous, varying by age, sex, region, season, and socioeconomic context. Verbal autopsy surveillance captures mechanisms such as drowning and poisoning frequently missed by facility-based systems. Integrating verbal autopsy data with socioeconomic and environmental information supports targeted prevention in climatically variable, resource-limited settings.

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SPATIAL PATTERNING AND SOCIAL DETERMINANTS OF INTENTIONAL INJURY MORTALITY IN BOTSWANA: A DISTRICT-LEVEL ANALYSIS OF NATIONAL FORENSIC RECORDS

Peter S. Larson[1], Mooketsi Molefi[2], Douglas J. Wiebe[1], Jaemin Jeon[3], Gormao Lee[1], Steven Shatani Mugoma[4]

[1]University of Michigan, Ann Arbor, MI, United States, [2]University of Botswana, Gabarone, Botswana, [3]Ann Arbor, MI, United States, [4]Botswana Police Service, Francistown, Botswana

Intentional injury mortality, including homicide, femicide, and suicide, is an understudied public health burden in sub-Saharan Africa. Social and environmental inequities are hypothesized to drive geographic clustering of these deaths, yet few studies have mapped their spatial distribution or tested ecological determinants using individual-level mortality data in this region. We analyzed forensic autopsy records from the Botswana Police Service Forensic Pathology Unit spanning 2018-2025, representing the most comprehensive intentional injury mortality dataset assembled for Botswana. Deaths were geocoded to district and aggregated to generate district-level incidence rates stratified by injury type and sex. Spatial clustering was assessed using local indicators of spatial autocorrelation. District-level covariates including urbanization, socioeconomic deprivation, population density, distance to health and police services, and climate variables were derived from census, survey, and gridded remote sensing

sources. Associations with mortality rates were estimated using spatial regression models. Analysis of an initial dataset of 2,225 forensic autopsies (2018-2022), comprising 1,479 homicides and 746 suicides, yielded national rates of 14.79 and 7.46 per 100,000, respectively. Men accounted for 88.2% of suicides, concentrated in the 18-45 age group; women comprised 37.3% of homicide victims across all age groups. Preliminary spatial analyses identified geographic clustering of intentional injury mortality with distinct patterns by injury type and sex. Analyses incorporating an extended dataset through 2025 and testing associations with social and environmental determinants are ongoing; full results will be presented. In conclusion, spatial analysis of national forensic records reveals substantial geographic inequity in intentional injury mortality in Botswana and identifies modifiable social and environmental determinants that can guide targeted prevention programming.

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BEYOND THE BINARY: LEVERAGING VERTICAL PROGRAMS FOR INTEGRATED ANTIMICROBIAL RESISTANCE CONTROL

Nochiketa Mohanty[1], Yemisi Ogundare[2], Silvia Kelbert[3], Soumeya Allawali Somdouanda Ouangraoua[4], Paa Kobina Forson[5], Peter Abah[2], Debadutta Parija[1], Lisa Noguchi[3], Rima Shretta[3], Aleisha Rozario[3], Abhishek Thakkar[1], Annick Raissa Ouelhore Sidibe[4], Adetiloye Oniyire[2], Virgile Madua Kikaya[6], Tigistu Adamu Ashengo[3]

[1]Jhpiego, New Delhi, India, [2]Jhpiego, Abuja, Nigeria, [3]Jhpiego, Baltimore, MD, United States, [4]Jhpiego, Ouagadougou, Burkina Faso, [5]Jhpiego, Accra, Ghana, [6]Jhpiego, Kinshasa, Democratic Republic of the Congo

Global antimicrobial resistance (AMR) caused 1.27 million deaths in 2019 and contributed to 4.95 million more, with projections of 10 million annually by 2050, largely in Asia and Africa. Vertical programs for tuberculosis, HIV, and malaria maintain strong drug-resistance surveillance systems that remain disconnected from national AMR platforms (e.g., GLASS, TRACSS) and from each other. Maternal-newborn-child health programs also operate in silos, addressing multidrug-resistant infections without coordinated responses. This fragmentation duplicates laboratory capacity, generates conflicting stewardship messages, and leaves critical data gaps. Integration is feasible and effective. In Nigeria, Jhpiego implemented an integrated quality improvement platform across malaria, TB, HIV, and MNCH services in 44 LGAs using WHO Point-of-Care QI and Plan-Do-Study-Act cycles. Rapid diagnostic test use increased to 99.7% in 2025 (from 96.9% in 2024), inappropriate ACT use fell from 1.2% to 0.04%, and antibiotic misuse among confirmed malaria cases dropped from 14% to 9%. Similarly, a baseline assessment in the Com-WATCH digital initiative across six states revealed fragmented data systems but strong feasibility for community surveillance: moderate AMR knowledge (61.4%), high positive attitudes (90.5%), variable practices (76.1%), high digital readiness (73.9%), and strong willingness (88.4%) among pharmacists and vendors. These findings support three priorities: (1) repurpose vertical platforms for broader AMR surveillance; (2) establish joint AWaRe-based stewardship committees; and (3) create unified dashboards integrating resistance and antimicrobial-use data. Operational pathways include cross-program training, pooled financing, and community engagement. Policymakers, program leaders, researchers, and donors must align efforts toward integrated systems. Leveraging vertical program strengths with One Health approaches can close critical gaps, optimize antibiotic use, and enable sustainable AMR control.

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REFLECTIONS ON FIFTEEN YEARS OF RESEARCH CAPACITY STRENGTHENING IN LOW- AND MIDDLE-INCOME COUNTRIES: THE NEXT GENERATION SCIENTIST PROGRAM

Chanelle Pillay[1], Edmund Ekuadzi[2], Helen Yifter[3], Carlos M. Cobos[4], Jenni Gray[5], Paul G. Ashigbie[6], Akiko N. Keller[5]

[1]Global Health, Novartis, Johannesburg, South Africa, [2]Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, [3]University of Rwanda, Kigali, Rwanda, [4]Hospital Alemán of Buenos Aires, Buenos Aires, Argentina, [5]Global Health, Novartis, Basel, Switzerland, [6]Biomedical Research, Novartis, Cambridge, MA, United States

Research capacity in low- and middle-income countries (LMICs) remains limited relative to disease burden, amplified by lack of training opportunities, poor infrastructure, and low retention of early career scientists. The Next Generation Scientist (NGS) program by Novartis in collaboration with the University of Basel, is a 3-month research fellowship designed to strengthen technical expertise, scientific leadership, and translational research capabilities among early-career scientists from LMICs. The program delivers training in drug discovery, clinical development, regulatory science and scientific communication, supported by individualized scientific mentorship and structured skills development modules. Since its inception in 2011, more than 260 fellows from over 30 countries have completed the program. In routine pre- and post-training evaluations, trainees reported substantial gains in scientific and professional skills including experimental design, data analysis, data interpretation, communication and others. Results from follow up program alumni surveys and case studies highlight three core impact domains: (1) scientific skill advancement evidenced by increased research output; (2) institutional capacity strengthening, demonstrated through expanded collaborations of beneficiary institutions, and the establishment of new clinical and laboratory services and (3) long-term workforce retention, with over 75% of alumni remaining scientifically active in their home countries. Key program constraints include cross-border research sample transfer restrictions, short duration for laboratory intensive projects, and the need to balance program scale with individualized mentorship. Virtual pre-program orientation, enhanced engagement with home supervisors, and clearer project scoping frameworks have partially mitigated some of these limitations. Fifteen years of experience show that initiatives like the NGS program can catalyze durable research capacity strengthening in LMICs, enabling locally relevant research, and supporting global health system strengthening priorities.

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STOCHASTIC QUANTITATIVE MICROBIAL RISK ASSESSMENT (QMRA) OF HAND-MEDIATED PATHOGEN EXPOSURES WITH CLINICAL VALIDATION IN HIGH-BURDEN DOMESTIC SETTINGS: MAPUTO, MOZAMBIQUE

Julia Sobolik[1], Mahira Amade[2], Cynthia Silva[2], Liliana Dengo-Baloi[1], Joseph Wells[1], Alesha Myers[1], Laura Braun[1], Oliver Cumming[1], Edna Viegas[3], Jacqueline Knee[1]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]Centro de Investigação e Treino em Saúde da Polana Caniço (CISPOC), Maputo, Mozambique, [3]Instituto Nacional de Saúde de Moçambique, Maputo, Mozambique

Domestic environments in low-resource settings often represent key enteric pathogen reservoirs. However, understanding specific exposure pathways and associated environmental risks is critical for targeted mitigation. This study quantified hand-mediated infection risks among children in Maputo, Mozambique using a stochastic quantitative microbial risk assessment (QMRA) model and validation with clinical stool data. Hand rinse samples (n=359) collected from children <24 months were concentrated and analysed for enteric pathogens using the TaqMan Array Card (TAC). Left-censored concentration data were fitted to log-normal distributions using Maximum Likelihood Estimation and incorporated into a QMRA model using established dose-response relationships and Monte Carlo simulations (n=10,000) to estimate *Cryptosporidium* and enterotoxigenic *E. coli* (ETEC) infection risks. Model performance was validated against matched stools collected seven days post-hand rinse using Area Under the Curve (AUC) analysis. Hand rinse prevalence was 11% (*Cryptosporidium*) and 5% (ETEC). The mean predicted hourly infection risk was 0.38 for *Cryptosporidium* (2.5th - 97.5th percentile: 0.08, 0.996) and 0.004 for ETEC (2.5th - 97.5th percentile: 0.0002, 0.021). Risk estimates did not significantly predict stool positivity (determined by TAC) for either pathogen (AUC: 0.49-0.50). Mean *Cryptosporidium* risk was nearly identical between stool-positive and negative children (37.8% vs. 37.2%), indicating ubiquitous faecal contamination in the domestic environment. This study

underscores the importance of validating QMRA models to contextualize risk, particularly in high-burden settings. The low predictive value highlights the transient nature of hand contamination and suggests transmission is driven by other, dominant pathways or impacted by high levels of acquired immunity not captured by standard dose-response models. Future validation efforts integrating concurrent pathways (caregivers' hands, weaning foods, and stored water) to fully characterize the domestic environment are warranted.

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ENVIRONMENTAL SURVEILLANCE AS A TOOL FOR MEASLES DETECTION: CORRELATION WITH CLINICAL CASES IN PAKISTAN

Wardah Mujahid[1], Farah Naz[2], Junaid Iqbal[3], Tahir Yousafzai[1], Sonia Qureshi[1], Furqan Kabir[2], Aneeta Hotwani[1], Zarfishan Hussain[1]

[1]Aga Khan University Hospital, Karachi, Pakistan, [2]Aga Khan University, Karachi, Pakistan, [3]Aga Khan University, karachi, Pakistan

Measles continues to be a major public health challenge in Pakistan, with repeated outbreaks largely driven by gaps in immunization coverage and delays in traditional surveillance systems. In this context, wastewater-based environmental surveillance offers a promising way to detect virus circulation at the community level, even before cases are reported. In this study, we assessed the usefulness of environmental surveillance for measles detection across three towns of Karachi between October 2023 and October 2024. A total of 1,759 wastewater samples from 32 sites were analyzed using the TaqMan Array Card platform, and weekly detection patterns were compared with reported clinical measles cases. Overall, a clear association was observed in Gulshan-e-Iqbal, while weaker or inconsistent patterns were seen in Gadap and Lyari. When we explored timing differences between environmental detection and reported cases, we found that accounting for site-specific delays strengthened the relationship across all areas. In a subset of samples, comparison with real-time RT-PCR showed that the TaqMan Array Card had high specificity but lower sensitivity. These findings suggest that environmental surveillance can provide early signals of measles circulation and, when interpreted with appropriate timing, can complement existing surveillance systems. This approach has the potential to strengthen outbreak detection and response in high-burden settings like Pakistan.

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ZOONOTIC ENTERIC PATHOGEN EXPOSURE IN CHILDREN: VALIDATION OF THE ENTEROPATHOGENS INDEX (FECEZ) IN MAPUTO, MOZAMBIQUE

Audrey Safir[1], Julia Sobolik[2], Mahira Amade[3], Cynthia Silva[3], Joseph Wells[2], April Ballard[4], Laura Braun[2], Oliver Cumming[2], Edna Viegas[5], Jacqueline Knee[2]

[1]Department of International Health, Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD, United States, [2]Department of Disease Control, Environmental Health Group, London School of Hygiene & Tropical Medicine, London, United Kingdom, [3]Centro de Investigação e Treino em Saúde da Polana Caniço (CISPOC), Maputo, Mozambique, [4]Population Health Sciences, Georgia State University, Atlanta, GA, United States, [5]Instituto Nacional de Saúde de Moçambique, Maputo, Mozambique

Enteric pathogen exposure, particularly from animal faeces, is a critical driver of child morbidity and mortality in Mozambique. Despite this burden of zoonotic pathogens, there are no standardized, field applicable tools to quantify children's exposure to animal-derived enteric pathogens in high-burden, low-resource settings. This study aimed to adapt and validate the FECEZ Enteropathogens Index, a novel survey-based tool, to measure zoonotic enteric pathogen exposures among children <24 months through environmental and behavioural pathways in low-income, unplanned communities in Maputo, Mozambique. The FECEZ index, previously developed in Ecuador, was culturally adapted to the Mozambican context, including locally relevant animals, and administered to caregivers of enrolled children (n=132). Principal components analysis (PCA) was used to identify key environmental (e.g., rodents, poultry, cats) and behavioural (e.g., child soil contact, direct animal contact) exposure domains, resulting in 11 and 9 components, respectively. These PCA-derived components were used to construct sub-domain and composite exposure scores (median [IQR]: 10 [5-15]). Higher FECEZ scores were associated with established predictors of animal exposure, including animal ownership (β = 1.94, 95% CI: 0.16-3.71) and observed presence of animal faeces (β = 4.98, 95% CI: 3.39-6.56). However, reliance on these conventional animal exposure proxies alone would have missed approximately 52% of the exposure variability captured by the index. Test-retest reliability demonstrated strong agreement (ICC = 0.89), consistent with prior validation in Ecuador. These findings demonstrate that the FECEZ Index is a scalable, reliable tool that can be adapted across diverse low-resource settings to generate high-resolution measures of zoonotic enteric pathogen exposure. By capturing substantial exposure heterogeneity missed by common binary exposure proxies, this approach improves exposure assessment and enables more precise targeting and evaluation of interventions aimed at interrupting animal-mediated enteric pathogen transmissions.

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CLIMATE VARIABILITY AND DIARRHOEA BURDEN IN COSTAL GHANA: DISTRICT LEVEL TIME-SERIES ANALYSIS

Delia Akosua Benewah Bandoh[1], Mawuli Dzodzomenyo[1], Ben Aikins[1], Michael Kwame-Biney[2], Duah Dwomoh[1], Ernest Kenu[3]

[1]Univeristy of Ghana School of Public Health, Accra, Ghana, [2]Univeristy of Ghana School, Accra, Ghana, [3]Univ of Ghana, Accra, Ghana

Diarrhoea remains a public health concern in low-and middle-income countries like Ghana, with a prevalence of 12% among children under five years. Coastal areas are vulnerable to climate-related health consequences such as diarrhoea. We examined the relationships between diarrhoea, temperature, rainfall, and humidity across three coastal districts in Ghana's Volta Basin over a thirteen (13) year period. We conducted an ecological time-series analysis in Anloga, Keta, and Ketu South using 468 district-month observations from DHIMS 2 (January 2012 - December 2024). Diarrhoea prevalence was defined as diarrhoea cases divided by total outpatient visits. Climate data, namely temperature, rainfall, and relative humidity, were obtained from TerraClimate. Data was analysed by multivariate linear regression, distributed lag models (0-3 months), and district-stratified analysis. Mean diarrhoea prevalence was 4.38% (SD 1.54%), highest in Anloga (5.33%) and lowest in Keta (3.68%). Prevalence declined by 0.11% per year ($p < 0.001$). Peaks occurred in May (4.97%) and June (5.33%), matching the rainy season. In the adjusted model (R^2 = 0.307), both rainfall (β = 0.003) and mean temperature (β = 0.150) were significant positive predictors ($p < 0.001$ and $p = 0.035$, respectively). Current temperature showed no effect, but delayed effects appeared at 1-month ($r = 0.173$), 2-month ($r = 0.285$), and 3-month ($r = 0.321$) lags (all $p < 0.001$). District patterns differed: temperature drove diarrhoea in Keta, while rainfall drove it in Anloga and Ketu South. After excluding the COVID-19 period, results remained the same. Rainfall and temperature are key drivers of diarrhoea in coastal Ghana. Temperature effects are delayed by up to three months. Local climate patterns vary by district, supporting the need for climate-informed disease monitoring, targeted WASH improvements, and integration of climate forecasts into district health planning.

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WHEN HEAT BECOMES RISK: NONLINEAR EFFECTS OF WEEKLY MAXIMUM TEMPERATURE ON CHILDHOOD DIARRHEA IN LIMA, PERU (2010-2019)

Luciana Rojas Granda[1], Stella Maria Hartinger[2], Yasna K. Palmeiro Silva[1]

[1]Latin American Center of Excellence on Climate Change and Health, Lima, Peru, [2]Univerisdad Peruana Cayetano Heredia, Lima, Peru

Climate change is increasing the frequency and intensity of extreme heat, yet its impact on enteric diseases in rapidly urbanizing tropical settings

remains insufficiently understood. This study examines the relationship between weekly maximum temperature and childhood diarrhea in Lima, Peru, a Latin American megacity characterized by pronounced climatic and socioeconomic heterogeneity. We conducted a time-series analysis of 1,093,213 diarrhea episodes among children under five across 46 districts over 520 weeks (2010-2019). Generalized additive models with a negative binomial distribution were applied to capture nonlinear exposure-response relationships, adjusting for long-term trends, seasonality, and district-level socioeconomic factors. We found a clear nonlinear association, with risk increasing at higher temperature levels. Using 23°C as a reference, incidence decreased at lower temperatures (IRR=0.70 at 16.6°C) and increased at higher temperatures (IRR=1.08 at 26.2°C and 1.15 at 30.9°C), indicating up to a 15% higher risk under extreme heat conditions. Marked spatial heterogeneity was observed, with district-specific effects exceeding 0.13 IRR per °C in highly vulnerable areas. These findings highlight the presence of temperature thresholds and unequal vulnerability within urban populations. As extreme heat intensifies, strengthening climate-sensitive surveillance and targeting high-risk districts will be essential to reduce the burden of childhood diarrhea.

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MODELING SHEDDING CONTRIBUTIONS TO WASTEWATER SEWERSHEDS FROM DYNAMIC POPULATIONS USING SIMULATED HUMAN MOBILITY DATA

Akshay Deverakonda[1], Hossein Amiri[1], Yuke Wang[2], Andreas Züfle[1]

[1]Emory University, Atlanta, GA, United States, [2]Atlanta, GA, United States

Integrating human mobility data into wastewater surveillance can inform outbreak response by accounting for dynamic population movement through wastewater catchment areas ("sewersheds") and identifying likely areas outside of sewersheds from where outbreaks originate. While existing methods allow for calculation of population-adjusted signals to account for temporal variation in sewershed populations across time, few methods account for spatial population variation by quantifying movement in and out of sewersheds. This study simulated how residents and non-residents contribute through defecation events ("shedding") to observed wastewater surveillance signals, and whether there are differences in model outputs under assumptions of either shedding at home and shedding both at home and outside. This study used the previously developed "Patterns of Life" agent-based model to simulate 10,000 agents across Fulton County, Georgia for one year, capturing movement between homes, workplaces, and restaurants. A hypothetical epidemic was introduced using an agent-based susceptible-exposed-infectious-recovered framework, and each agent shed at one or more places they visited during the simulation. Eight sewershed boundaries from prior literature were incorporated to calculate the longitudinal numbers of shedding events and pathogen counts, shed by both residents and non-residents, into each sewershed over the course of the simulation. Additionally, the outputs of two sets of simulations, one with shedding only at agents' homes, and one with shedding at home and outside, were calculated to estimate the differences in model outputs through integrating an assumption of shedding outside the home. This simulation framework can be used to improve interpretation of wastewater surveillance data, especially from sewersheds with non-residential land use. It also provides a platform for other researchers to validate epidemiologic inference methods involving wastewater surveillance.

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DOES EXPOSURE TO ANIMALS AFFECT THE INFANT GUT MICROBIOME? CASE STUDY OF 6-18 MONTH OLD CHILDREN IN ESMERALDAS PROVINCE, ECUADOR

Viviana Alban[1], Kelsey Jesser[1], April M. Ballard[2], Caitlin Hemlock[1], Christine S. Fagnant-Sperati[1], Janet K. Hatt[3], Erica Fuhrmeister[1], Joan A. Casey[1], Gwenyth O. Lee[4], Gabriel Trueba[5], Joseph Eisenberg[6], Konstantinos T. Konstantinidis[3], Karen Levy[1]

[1]University of Washington, Seattle, WA, United States, [2]Georgia State University, Atlanta, GA, United States, [3]Georgia Institute of Technology, Atlanta, GA, United States, [4]Rutgers University, New Brunswick, NJ, United States, [5]Univ San Francisco Quito, Quito, Ecuador, [6]Univ of Michigan School of Public Health, Ann Arbor, MI, United States

In many low- and middle-income countries domestic animals are closely integrated into household environments, increasing opportunities for microbial exchange between animals and household members, including infants. However, the influence of animal exposure on the infant gut microbiome in these settings has not been well-studied, particularly in early developmental stages. We analyzed data from a longitudinal birth cohort in Ecuador, to examine associations between animal exposure and infant gut microbiome outcomes during the first 18 months of life. Infant stool samples collected at 6, 12, and 18 months were characterized using 16S rRNA sequencing. Animal exposure was assessed using animal pathway-specific metrics, including animal ownership, number of animals owned, presence of animals or their feces in household environments, and maternal or infant contact with animals, as well as a composite score that integrated all metrics. We used regression models to evaluate both cross-sectional and 6-month lagged associations with Shannon alpha diversity, Bray-Curtis beta diversity, and taxonomic abundances. We found that higher animal exposure scores at 12 months were associated with greater Shannon index both cross-sectionally (B=0.18; 95% CI:0.01-0.37) and at 18 months (B=0.22; 95% CI:0.001-0.44). Exposures involving animals or animal feces inside the household and maternal-animal contact were most closely aligned with alpha diversity patterns in the same time windows. Shifts in beta diversity with varying animal exposure score and pathway-specific metrics were not evident (PERMANOVA: p> 0.05). Number of animals owned showed the strongest associations with microbiome taxa at multiple windows, including increased abundance of both potentially beneficial taxa (e.g., *Oscillospiraceae* and *Ruminococcaceae*) and those associated with potential dysbiosis (e.g., *Enterobacteriaceae*). These findings suggest that animal exposures may shape infant gut microbiome development through both immediate and sustained effects, and that they potentially introduce microbes with both beneficial and detrimental functions.

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DECIPHERING THE TEMPERATURE FLUCTUATION IMPACT ON *PLASMODIUM FALCIPARUM* TRANSMISSION IN THE CONTEXT OF CLIMATE CHANGES

Dari F. Da[1], Joseph D. Challenger[2], Fatoumata Cissé[1], Inès G.L. Paré[1], Bernard M. Somé[1], Nicaise D. C. Djègbè[1], Thomas S. Churcher[2], Roch K. Dabiré[1]

[1]Institut de Recherche en Sciences de la Santé, Bobo-Dioulasso, Burkina Faso, [2]Imperial College London, London, United Kingdom

Two important entomological processes are involved in the malaria transmission in endemic countries: the vector's ability to reproduce in a particular environment and the pathogen's capability to develop within mosquito. Both *Anopheles*mosquito lifespan and *Plasmodium* sporogonic development depend on temperature and relative humidity, making it unclear how these processes will be influenced by climate change. Here, we investigated the role of temperature in malaria transmission using the wild vectorial system of *Plasmodium/ Anopheles* under climatic conditions closed to natural setting. Environmental parameters emulating Burkina Faso weather were generated inside incubators to simulate climatic periods throughout the year, including potential climate change scenarios. Mosquito Membrane Feeding Assays were performed to explore how climatic factors can affect *Plasmodium* sporogonic development. Wild *Anopheles coluzzii* females were fed on gametocyte-infected blood from asymptomatic individuals and were exposed in the simulated climatic conditions. Parasite infection levels were evaluated in mosquitoes of different conditions, detecting *Plasmodium* oocyst and sporozoite by microscopic dissection and molecular analysis. Twenty-five experiment replicates were performed using different gametocyte donors to include a variety of natural variables. Various infection levels were recorded according to the climatic conditions. It revealed that temperature variations partially or totally blocked parasite sporogonic development. The parasite inhibition occurs within 12 hours post-blood meal (during the early sporogony). Climatic conditions with

constant temperatures (in 25 and 33 °C interval) generated *Plasmodium* optimal development. Results suggest the optimal environmental conditions for parasite development in Burkina Faso simulated in the incubator was the period between August-September characterized by the relatively stable temperature. The work highlights the importance of temperature-dependence of *Plasmodium* sporogonic development and underlines the potential impact of climate change on malaria.

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THE CART BEFORE THE HORSE: GAMETOCYTE DEVELOPMENT PROTEIN 1 IS REQUIRED FOR TRANSCRIPTION OF ITS ANTISENSE LNCRNA (GDV1-AS)

Surendra K. Prajapati[1], Jeffrey X. Dong[1], Kim C. Williamson[2]

[1]Department of Microbiology and Immunology, Uniformed Services University of the Health Sciences, Bethesda and Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc., Bethesda, USA, Bethesda, MD, United States, [2]Department of Microbiology and Immunology, Uniformed Services University of the Health Sciences, Bethesda, USA, Bethesda, MD, United States

The regulation of the transition from asexual replication to sexual differentiation in human malaria parasites remains unclear, despite much speculation. *Plasmodium falciparum* gametocyte development protein 1 (GDV1) is a key upstream activator, inducing expression of *ap2-g*, which encodes the transcription factor AP2-G required for gametocytogenesis. The antisense long noncoding RNA (*gdv1-as*) has been proposed as a negative regulator of GDV1 and sexual differentiation; however, the factors controlling its expression and its mechanism of action remain unclear. To address these questions, we performed time-course analyses using transgenic reporter lines that quantitatively track GDV1 and AP2-G expression, alongside transcriptional profiling of *gdv1-as* and *ap2-g*, under GDV1 wild-type and knockdown conditions. Contrary to prevailing models, *gdv1-as* transcription followed GDV1 expression and coincided with *ap2-g* activation, emerging prior to detectable AP2-G protein. Notably, *gdv1-as* transcription was abolished in the absence of GDV1, mirroring *ap2-g* expression dynamics. Consistent with this, *gdv1-as* levels were reduced upon GDV1 knockdown but remained unchanged following AP2-G depletion, demonstrating that *gdv1-as* transcription is GDV1-dependent and downstream of GDV1 activity. These observations prompted us to test whether *gdv1-as* negatively regulates *ap2-g* expression to repress sexual differentiation. Disruption of *gdv1-as* did not alter *ap2-g* transcript levels. However, it resulted in a dramatic increase in AP2-G protein abundance and the proportion of AP2-G-positive schizonts. This strongly indicates that *gdv1-as* playing a role in the translational repression of *ap2-g*. Together, these findings redefine the GDV1-*gdv1-as* relationship and support a model in which GDV1 induces *gdv1-as*, which in turn fine-tunes sexual commitment by restricting AP2-G translation. **Disclaimer:** The authors have no conflicts of interest and the opinions expressed herein are those of the authors and do not reflect official policy.

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IN SITU VISUALIZATION OF THE MITOCHONDRION IN *PLASMODIUM FALCIPARUM* BY AN INTEGRATED CRYOGENIC CORRELATIVE LIGHT AND ELECTRON MICROSCOPY (CRYO-CLEM) APPROACH

Ijeoma C. Okoye[1], Yee-Wai Cheung[2], Shrawan K. Mageswaran[2], Manish Sharma[1], Karen Malanda[1], Joanne M. Morrisey[1], Yi-Wei Chang[2], Akhil B. Vaidya[1]

[1]Drexel University, Philadelphia, PA, United States, [2]University of Pennsylvania, Philadelphia, PA, United States

Malaria is a pressing global health challenge, causing about 200 million infections and 600,000 deaths annually. Due to the waning efficacy of frontline antimalarials, there is an increasing need to identify new antimalarial drug targets. Although the mitochondrion of malaria-causing *Plasmodium falciparum* parasites is a validated target of currently used antimalarial drugs, it remains an enigmatic organelle with a significant knowledge gap in the visualization of its native features and structural determination of its components including essential membrane protein complexes. The goal of this study was to generate unprecedented 3-dimensional visualizations of the native mitochondrial structural landscape within the cellular context of *P. falciparum* parasites to identify and determine novel mitochondrial structures. We employed an integrative *in situ* cryo-correlative light-electron imaging workflow involving vitrification of parasitized red blood cells, cryo-confocal imaging, cryo-focused ion beam milling and scanning electron microscopy (cryo-FIBSEM), and cryo-electron tomography (cryo-ET). Here, we have generated 3D visualizations of the asexual *P. falciparum* mitochondrion in its near-native state, highlighting features such as mitochondrion inner-outer membrane contact sites as well as the mitochondrion-endoplasmic reticulum contact sites (MERCS). Additional experiments involve *in situ* labelling of specific mitochondrial membrane complexes in isolated mitochondrial fragments and sub-tomogram averaging to resolve the structures of critical mitochondrial machineries to gain mechanistic insights. These studies are aimed at informing future antimalarial drug designs.

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SHORT-TERM *EX VIVO* CULTURE RESHAPES DEVELOPMENTAL COMPOSITION AND SELECTIVELY FILTERS *PLASMODIUM VIVAX* HAPLOTYPES

Beka R. Abagero[1], Franck Dumetz[2], Colby T. Ford[3], Tassew Tefera Shenkutie[4], Jean Popovici[5], Delenasaw Yewhalaw Y. Gebre[6], David Serre[7], Eugenia Lo[8]

[1]Drexel University, Philadelphia, PA, United States, [2]University of Maryland, Baltimore, Baltimore, MD, United States, [3]University of North Carolina at Charlotte, Chrlotte, NC, United States, [4]Tassew, Philadelphia, PA, United States, [5]Institut Pasteur of Cambodia, Phnom Penh, Cambodia, [6]Jimma University, Jimma, Ethiopia, [7]University of Maryland School of Medicine, Baltimore, MD, United States, [8]Philadelphia, PA, United States

Plasmodium vivax (*Pv*) infections are developmentally asynchronous and often polyclonal, complicating interpretation of bulk parasite transcriptomes. Here, we analyzed paired *in vivo* and short-term *ex vivo* transcriptomes from Ethiopian clinical isolates using stage deconvolution and *PvMSP*1 haplotyping. *Ex vivo* maturation modestly increased inferred schizont representation while largely preserving trophozoite and gametocyte signals. After adjustment for parasite stage composition, *in vivo* and *ex vivo* transcriptomes remained globally similar, with no genes differentially expressed at FDR<0.05, indicating the absence of a shared culture-induced transcriptional response. In contrast, short-term culture reduced multiplicity of infection, contracted within-host haplotype diversity, and non-randomly depleted specific haplotypes, consistent with a clonal bottleneck. In a subset of low-complexity infections, residual expression patterns were clustered by dominant haplotype, suggesting genotype-associated transcriptional heterogeneity independent of developmental stage. Together, these findings indicate that short-term *ex vivo* culture enriches late asexual stages and selectively filters pre-existing clones rather than inducing a common transcriptional program. These results emphasize the need to explicitly model developmental composition and infection complexity when interpreting *Pv* transcriptomes from natural infections.

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GENETIC BASIS OF HEMOGLOBINOPATHY-ASSOCIATED OXIDATIVE STRESS ADAPTATION AND ARTEMISININ TOLERANCE OF THE HUMAN MALARIA PARASITE *PLASMODIUM FALCIPARUM*

Chiara Micchelli[1], Camilla Valente Pires[1], John H. Adams[2]

[1]University of South Florida, Tampa, FL, United States, [2]University of South Florida Coll of Pub Hlth, Tampa, FL, United States

Malaria continues to pose a major global health burden, causing over 280 million infections and more than 600,000 deaths in 2024, largely due to *Plasmodium falciparum*. In malaria-endemic sub-Saharan Africa, hemoglobinopathies like sickle cell trait create pro-oxidative red blood cell environments that limit parasite growth. Oxidative stress is also central to the mechanism of action of artemisinin (ART) drugs, the cornerstone

of frontline antimalarial therapy. Despite mechanistic and geographical commonality between sickle cell trait and ART, the effects of oxidative stress on parasite adaptation and ART efficacy are not well-described. Given the shared role of oxidative stress in both hemoglobinopathies and ART action, we hypothesized that adaptation to hemoglobinopathy-associated oxidative stress increases parasite tolerance to ART through alterations in growth and gene expression. We used a physiologically and morphologically relevant *in vitro* model that mimics the sickle trait oxidized microenvironment and examine how hemoglobinopathy-associated oxidative stress affects parasite growth and drug sensitivity. Parasites grown in oxidized red blood cells exhibited higher mean IC_{50} values for dihydroartemisinin (DHA) and displayed slower growth marked by a delayed transition from the ring stage to the trophozoite stage. Transcriptional analysis of oxidative stress-exposed rings pointed to significant changes in translation, export, apicoplast, trafficking, and mitochondrial pathways. These findings suggest that hemoglobinopathy-associated oxidative stress reshapes parasite physiology and transcriptional programs, with potential consequences for ART efficacy in malaria-endemic regions.

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DIFFERENTIAL EXPRESSION OF THE PHIST MULTI-GENE FAMILY IN SEVERE MALARIA: A CASE-CONTROL STUDY

Kent Hardart[1], Jonathan Lawton[1], Emily Stucke[1], Drissa Coulibaly[2], Rafal Sobota[1], Jean-Paul Courneya[1], Savy M. Sebastian[1], Sarika Kapadia[1], Matthew Adams[1], James B. Munro[3], Ankit Dwivedi[3], Abdoulaye K. Kone[2], Karim Traore[2], Boureima Guindo[2], Bourama M. Tangara[2], Amadou Niangaly[2], Modibo Daou[2], Issa Diarra[2], Youssouf Tolo[2], Mody Sissoko[2], Bryan Evan Cummings[1], Matthew B. Laurens[1], Amed Ouattara[1], Bourema Kouriba[2], Ogobara K. Doumbo[2], Shannon Takala Harrison[1], Christopher V. Plowe[1], Joana C. Silva[3], Mahamadou A. Thera[2], Mark A. Travassos[1]

[1]Malaria Research Program, Center for Vaccine Development and Global Health, University of Maryland School of Medicine, Baltimore, MD, United States, [2]Parasites & Microbes Research and Training Center, University of Sciences, Techniques and Technologies, Bamako, Mali, [3]Institute for Genome Sciences, University of Maryland School of Medicine, Baltimore, MD, United States

Severe malaria syndromes including cerebral malaria (CM), severe malarial anemia (SMA), and concurrent CM + SMA cause significant morbidity and mortality in sub-Saharan African children. A central pathogenic mechanism is sequestration, whereby parasite-infected red blood cells (iRBCs) adhere to vascular endothelium and evade splenic clearance. Adhesion of iRBCs to cerebral vasculature is a key feature of CM and thought to be mediated by parasite virulence factors encoded by multigene families. One such family, the *phist* parasite multigene family, is comprised of a, b, and c subfamilies, each with distinct but incompletely characterized functions. PHISTb proteins mediate erythrocyte remodeling, and PHISTc proteins facilitate protein export. The function of PHISTa proteins is unknown. Because PHISTb proteins localize to the iRBC membrane and may mediate cytoadherence to the endothelium, we hypothesized that this subfamily is upregulated in CM and CM + SMA. We also hypothesized that PHISTa and PHISTc genes are differentially expressed in severe malaria compared to matched, uncomplicated malaria controls. We compared gene expression between Malian children with severe malaria (CM, SMA, or CM + SMA) and matched controls with uncomplicated disease. Controls were matched by age, class, residence, sex, and ethnicity. Transcripts were identified from RNA sequencing data using a nucleotide BLAST pipeline. We found upregulation of PHISTb transcripts in CM cases compared with matched controls ($n = 14$, $p = 0.025$) but did not find differential expression of PHISTb in CM + SMA cases ($n = 7$, $p = 0.69$). In contrast, PHISTa transcripts were downregulated in SMA cases compared with matched controls ($n = 12$, $p = 0.042$). PHISTc transcripts were not differentially expressed in any comparisons. These findings support a role for differential PHISTb expression in CM pathogenesis, consistent with our hypothesis. Upregulation of PHISTb transcripts and the potential mechanistic link between PHISTb proteins and cerebrovascular sequestration suggests a promising target for future therapeutic and prophylactic interventions against cerebral malaria.

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ASSOCIATIONS BETWEEN GUT MICROBIOTA-DEPENDENT BILE ACID METABOLISM AND SUSCEPTIBILITY TO SEVERE MALARIA

Ayana Baidya, Sawyer B. Lehman, Rafael B. Polidoro, Nathan W. Schmidt

Ryan White Center for Pediatric Infectious Diseases and Global Health, Herman B Wells Center for Pediatric Research, Department of Pediatrics, Indiana University School of Medicine, Indianapolis, IN, United States

Increasing evidence indicates that the gut microbiota influences malaria severity by modulating host immune responses to *Plasmodium* infection. Bile acids are host derived metabolites that are further modified by the gut microbiota and are emerging as important regulators of immune function. However, the role of bile acids in shaping immune responses to severe malaria remains poorly understood. Previous studies from our lab demonstrated that mice harboring different gut microbial composition exhibit differential susceptibility to severe malaria and had varied bile acid profiles. We hypothesize that gut microbiome-dependent alterations of bile acids contribute to differential host immune response to severe malaria. To test this hypothesis, we performed targeted metabolomic profiling of bile acids in the plasma and cecal content of groups of mice susceptible and resistant to severe malaria at baseline and during *Plasmodium* infection. Susceptible mice exhibited significantly higher levels of unconjugated bile acids at baseline compared to the resistant mice, while the total bile acid levels remain comparable between the two groups. Ongoing research is directed towards demonstrating the sufficiency of these bile acids to modulate the host immune response to *Plasmodium* and consequently the severity of malaria. Additional studies are underway to investigate this bile acid-microbiome interaction in African children with severe malaria compared to community controls. As bile acids can act as ligands to different receptors and modulate the function or differentiation potential of immune cells, they may have functional immunological consequences in severe malarial outcome. These findings are poised to identify a molecular mechanism by which the gut microbiome affects the severity of malaria.

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HIGH BURDEN OF CEREBRAL HYPOXIA DETECTED BY FREQUENCY-DOMAIN NEAR-INFRARED SPECTROSCOPY IN UGANDAN CHILDREN WITH SEVERE MALARIA

Michael D. Lintner Rivera[1], Collins Akugizibwe[2], Edrine Katumba[2], Kagan A. Mellencamp[1], Irene Bagala[2], Nicole O'Brien[3], Andrea Conroy[1], Ruth Namazzi[4], Hans Ackerman[5], Chandy C. John[1]

[1]Ryan White Center for Pediatric Infectious Disease and Global Health, Indiana University School of Medicine, Indianapolis, IN, United States, [2]Global Health Uganda, Jinja, Uganda, [3]Nationwide Children's Hospital, The Ohio State University, Columbus, OH, United States, [4]Makerere University, Kampala, Uganda, [5]National Institutes of Health, Rockville, MD, United States

Severe malaria is a leading cause of pediatric mortality and is frequently complicated by cerebral dysfunction, yet direct assessment of brain oxygenation is not routinely available. Systemic markers such as oxygen saturation (SpO_2) and lactate may not reflect regional tissue hypoxia. We used frequency-domain near-infrared spectroscopy (fdNIRS) to characterize cerebral regional tissue oxygenation (rSO_2) in children hospitalized with severe malarial anemia at Nalufenya Children's Hospital in Jinja, Uganda. Serial measurements were obtained and underwent strict quality control, with <5% of studies excluded due to inadequate signal. Among 32 participants with high-quality data, a strikingly high burden of cerebral hypoxia was observed, with approximately 90% demonstrating low rSO_2 (<55%) at presentation. Following transfusion at enrollment, approximately 70% remained hypoxic, suggesting persistent impairment in cerebral

oxygenation despite achieving current guideline-directed transfusion thresholds (hemoglobin >5 g/dL). In the overall cohort, including children with cerebral malaria and severe malarial anemia, SpO_2 showed a moderate correlation with rSO_2 (Spearman's ρ=0.55, p<0.001); subgroup-specific analyses within SMA are ongoing. No associations were observed with lactate. These findings suggest that systemic measures, including hemoglobin, SpO_2, and lactate, incompletely capture cerebral oxygenation. fdNIRS was feasible in this setting and demonstrated a high prevalence of previously unrecognized cerebral hypoxia. Persistent cerebral hypoxia despite transfusion may represent a potentially modifiable contributor to morbidity and mortality in severe malarial anemia and highlights the importance of tissue-level monitoring to improve risk stratification and guide targeted interventions.

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MULTI-OMICS ANALYSIS IDENTIFIES DISRUPTION OF NOX2-DEPENDENT NETOSIS IN SEVERE MALARIAL ANEMIA

Sharley A. Wasena[1], Qiuying Cheng[2], Clinton Odhiambo Onyango[3], Shamim Wanjiku Osata[4], Ivy Hurwitz[5], Collins Ouma[6], Kristan Schneider[7], Philip Seidenberg[8], Benjamin McMahon[9], Samuel Anyona[5], Douglas Jay Perkins[2]

[1]Maseno University, Kisumu, Kenya, [2]University of New Mexico, Albuquerque, NM, United States, [3]Maseno University, Albuquerque, NM, United States, [4]University of New Mexico-Kenya Global Health Programs, Nairobi, Kenya, [5]Univ of New Mexico, Albuquerque, NM, United States, [6]Maseno Univ, Kisumu, Kenya, [7]UNM, Albuquerque, NM, United States, [8]University o, Albuquerque, NM, United States, [9]Los Alamos National Laboratory, Los Alamos, NM, United States

Severe malarial anemia [SMA; hemoglobin (Hb) <6.0 g/dL] caused by *Plamodium falciparum* remains a major cause of morbidity and mortality in children under five. Neutrophils defend against infection through phagocytosis, degranulation, and NET formation (NETosis). However, dysregulated NETosis contributes to the pathogenesis of inflammatory and infectious diseases. To investigate mechanisms underlying dysregulated NETosis in SMA, we characterized NETosis-related gene (NRG) expression using whole-blood transcriptomics from children with non-SMA (Hb ≥6.0 g/dL, n=41) and SMA (n=25) at admission (pre-Rx, day 0) and convalescence (post-Rx, day 14). Differentially expressed genes (DEGs) in SMA were determined using edgeR [$|\log_2 FC|>0.585$; FDR-adjusted p-value<0.05]. BloodGen3 analysis revealed dysregulated neutrophil activity, and pathway enrichment using MetaCore™ identified NETosis in Systemic Lupus Erythematosus as the top pathway in SMA (padj =2.235E-07). Key NOX2-dependent NETosis effectors were downregulated [FCGR2A (-0.70), MAPK14 (-0.78), PRKCD (-0.61), NCF1/p47-phox (-0.73), NCF2/p67-phox (-0.61), PADI4 (-1.10), and IL1B (-1.51)]. Upregulated DEGs included DEFA1 (+1.45), MAPK3/ERK1 (+0.60), PRKCE (+0.66), ELANE (+1.01), HIST1H1C +1.10, HIST1H2AC +1.04, and HIST1H1B +0.71. Downregulated networks were enriched for inflammation, while upregulated networks were associated with a disrupted signaling cascade that drives chromatin remodeling. Further analysis revealed that NETosis pathway remained significantly enriched (padj=1.39×10E05) at day 14, though no individual genes met DEG thresholds, indicating persistent transcriptional perturbation. These findings were validated in a subset of children with non-SMA (n=19) and SMA (n=16) using SomaLogic proteomic platform, and in a separate non-SMA (n=38) and SMA (n=12) cohort using Illumina HumanHT-12 v4 BeadChip array. This study provides the first multi-omics characterization of NETosis in SMA, demonstrating sustained dysregulation of NOX2-dependent pathways, identifying NETosis as a potential target for immunotherapeutic intervention.

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TRIAZINE-CORE MMV007808 DERIVATIVES AS NEW DHODH-INHIBITING ANTIMALARIAL

Kevin A. Kunz[1], Leticia Do Amaral[2], Yuexin Li[3], Dejan Nikolic[1], Emilio Merino[2], Jane X. Kelly[4], Max Totrov[5], M. Belen Cassera[2], Paul R. Carlier[1]

[1]University of Illinois Chicago, Chicago, IL, United States, [2]University of Georgia, Athens, GA, United States, [3]Portland State Univ, Portland, OR, United States, [4]Portland VA Medical Ctr, Portland, OR, United States, [5]Molsoft LLC, San Diego, CA, United States

Malaria remains a major global health challenge, with the World Health Organization (WHO) reporting over 250 million cases and approximately 600,000 deaths in 2023. The spread of resistance to artemisinin-based combination therapies shows the urgent need for novel drug targets and new classes of antimalarials. To accelerate discovery, the Medicines for Malaria Venture (MMV) developed the Malaria Box, a curated collection of 400 chemically diverse, drug-like and probe-like compounds active against *Plasmodium falciparum*. Within the Malaria Box we were drawn to MMV007808, due to its structural similarities to another chemotype in our lab. Against the multi-drug-resistant Dd2 strain of *P. falciparum*, this triazine-core compound inhibited growth with EC_{50} = 466 ± 80 nM. We then prepared number of analogs featuring variation of the aniline substituent. Illustrative examples **1** and **2,** featuring 4-CF_3 and 4-SF_5 substituents, showed similar and improved potency respectively. To assess the potential antimalarial target of PRC2522, a public database of antimalarial HTS hits was computationally screened for similarity to **1** using Atomic Property Fields (APF) method in ICM Pro. This screen gave [1,2,4] triazolo[1,5-*a*]pyrimidines (e.g. DSM338, DSM265) as top matches. These compounds overlay well with **1** and **2**, each possessing two ring systems, two hydrogen bond donors and two hydrogen bond acceptors. Since the DSM antimalarials are known to target *Plasmodium* dihydroorotate dehydrogenase (DHODH), a key enzyme for pyrimidine biosynthesis we undertook further studies. First, compounds were tested against a cytosolic DHODH-expressing strain, which is insensitive to traditional inhibitors. As expected, **2** and DSM265 both were much less potent against this strain. Second, resistance selection studies with PRC2522 and DSM265 successfully yielded resistant clones. Cross-resistance studies of these clones with the compounds confirmed that DHODH is the target of PRC2522. Lastly, we will disclose ADME, metabolite ID, and in vivo efficacy studies with **2.**

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DISCOVERY AND DEVELOPMENT OF PFGARP TARGETING ANTI-MALARIALS

Tanbir Najrana[1], Hannah Wu[1], Brajesh Kumar Singh[1], Alan Ardito[1], Aidan Biondi[1], Lilliana Hammons[1], Michael Chan[1], Brooke Skinner[1], Kanika Men[1], Samantha Maguire[1], Nastassja Matus[1], Ximenna G. Rivera[1], Teja Prakash[1], Yannick Johnson[1], Nazrin Rustamzade[1], Pratima Gurung[2], Camille Hebert[1], Emilia Ferraro[1], Sunthorn Pond-Tor[1], Rose Guilfoyle[1], Benjamine Moore[1], Ian Petrow[1], Audrey Kertscher[1], Jacqueline Real[1], Shira Wolpowitz[1], Aristotle Apostolakis[1], Sovanny Pond-Tor[1], Edwin Mwakio[3], Douglas O. Ochora[3], Farid A. Salim[3], Ashley Bunnel[4], Ashley Simkovitch[5], Matthew Araujo[5], Irina Oleinikov[5], Sachy Orr-Gonzales[6], Tarik C. Ouahes[6], Lynn E. Lambert[6], Mandar Naik[1], Dipak K. Raj[7], John Michael Ong'echa[3], Hoseah Akala[8], Patrick Duffy[6], Adel Nefzi[4], Andrew V. Oleinikov[5], **Jonathan D. Kurtis**[1]

[1]Brown University Medical School, Providence, RI, United States, [2]Harvard Medical School, Boston, MA, United States, [3]Kenya Medical Research Institute, Kisumu, Kenya, [4]Florida International University, Port St. Lucie, FL, United States, [5]Florida Atlantic University, Boca Raton, FL, United States, [6]National Institutes of Health, Bethesda, MD, United States, [7]University of South Florida, Tampa, FL, United States, [8]USAMRU-K, Kisumu, Kenya

The spread of artemisinin resistant parasites underscores the urgent need to identify novel anti-malarial drugs. PfGARP, a vaccine candidate, also represents a high value druggable target based on: 1) its surface expression

on iRBCs, 2) its absence of homology with host proteins, 3) its limited sequence variation, and 4) the ability of monovalent anti-PfGARP antibody to rapidly kill parasites. We screened a 30M compound, combinatorial small molecule library (CTS-FIU) to identify compounds that inhibit binding of anti-PfGARP to rPfGARP. We reason that compounds which bind to the same region of PfGARP targeted by parasite-lethal anti-PfGARP antibodies will be enriched for effective PfGARP targeting anti-malarials. Using our positional scanning method, we identified 36 structurally related compounds which kill *Plasmodium falciparum* parasites with IC_{50} < 200 nM. Our lead compound, 2741-19 has an IC_{50} of 25-100 nM for killing 3D7 strain parasites, multidrug/artemisinin resistant parasites, and freshly collected field isolates and has a minimum inoculum for resistance (MIR) > $5*10^9$. In drug-pulse experiments, complete killing of trophozoite stage parasites was achieved after 3 hours of exposure to 500 nM 2741-19, qualifying this compound as a fast-acting antimalarial. 2741-19 has a selectivity index of 500-1000 comparing the IC_{50} for killing parasites versus multiple human cell lines and shows no mutagenicity or genotoxicity in standard toxicologic assays. 2741-19 binds recombinant PfGARP and native PfGARP in parasite extracts by ELISA and 2D-NOSY NMR. Treatment of parasites with 2741-19 leads to upregulation of protein ubiquitination, proteasome inhibition and apoptosis- features concordant with anti-PfGARP antibody treatment. 2741-19 is freely soluble in aqueous buffers and, in non-human primates, has a distribution $t_{1/2}$ of 1.4 hours and an elimination $t_{1/2}$ of 38 hours. When dosed subcutaneously at 5mg/kg, 2741-19 rapidly cleared parasites in the *P falciparum*/NSG mouse model. We are currently evaluating the efficacy of 2741-19 in the *Aotus/P. falciparum* non-human primate model.

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DRUG REPURPOSING FOR MALARIAL COMBINATION THERAPY

Awet Alem Teklemichael, Jian Wu, Mamadou Sangare, Fangzheng Xu, Stadler Rachel, Hans Ackerman, Xinzhuan Su
NIH/NIAID, Rockville, MD, United States

Drug resistance to currently available antimalarial therapies is one of the major obstacles to malaria elimination. Although artemisinin-based combination therapies (ACTs) have been highly effective, delayed parasite clearance to ACTs has been widely reported in Southeast Asia and, more recently, in parts of Africa and South America. Chloroquine (CQ) is affordable and safe antimalarial drug; however, CQ resistance (CQR) *Plasmodium falciparum* (*Pf*) parasites have been reported in all malaria endemic regions. Various compounds have been shown to reverse CQR and could be explored for combination therapies with CQ. CQR is primarily mediated by mutations in the *Pf* CQ resistance transporter (PfCRT). Targeting parasites with CQ plus compounds that specifically kill parasites with mutant PfCRT may limit their capacity to develop further resistance. In our previous work, we identified several existing compounds with activity against multiple *Pf* isolates carrying mutant PfCRT. In this study, we performed molecular docking simulations to identify compounds with high binding affinity for PfCRT and evaluated their activity against both CQ-sensitive and CQ-resistant strains. Selected compounds with strong predicted affinity were tested in combination with CQ *in vitro*, demonstrating synergistic effects and reduced CQ IC_{50} values when used alongside sub-inhibitory (IC_{25}) concentrations of these compounds. Overall, our findings highlight the potential of repurposed drugs as partner therapies with CQ, offering a promising approach to overcoming resistance and enhancing antimalarial efficacy.

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A PILOT STUDY OF DIHYDROARTEMISININ-PIPERAQUINE VERSUS QUININE FOR *PLASMODIUM MALARIAE* AND *P. FALCIPARUM* MALARIA: PARASITE CLEARANCE KINETICS AND ANEMIA RECOVERY

Dao Francois[1], Siaka Goita[2], Aboubacrin S. Haidara[1], Ousmaila Diakite[1], Mohamed Maiga[3], Fatoumata Ousmane Maiga[4], Fanta Sogore[5], Fatoumata O. Diallo[1], Abdoulaye Djimde[6], Laurent Dembele[7]
[1]PMRTC-USTTB, Bamako, Mali, [2]MRTC, Bamako, Mali, [3]Université Des Sciences Des Techniques Et Des Technologies de Bamako (USTTB), Bamako, Mali, [4]University of Sciences, Techniques and Technologies of Bamako, Bamako, Mali, [5]MRTC, BAMAKO, Mali, [6]MRTC-USTTB, Bamako, Mali, [7]University of Science Technical and Technology of Bamako, Bamako, Mali

Plasmodium malariae is a neglected parasite causing chronic infections and long-term morbidity, including nephropathy and anemia. Treatment strategies for *P. malariae* remain poorly defined, extrapolated from *P. falciparum* studies. We conducted a pilot study comparing dihydroartemisinin-piperaquine (DHA-PPQ) versus quinine for *P. malariae* and *P. falciparum* infections, focusing on parasite clearance kinetics and anemia recovery. Patients with uncomplicated malaria (*P. falciparum* or *P. malariae*) were randomized to DHA-PPQ or quinine. Parasite clearance was monitored by serial microscopy; hemoglobin was measured at baseline and during 28-day follow-up. DHA-PPQ achieved significantly faster parasite clearance than quinine in both species. For *P. malariae*, DHA-PPQ produced a steep parasitemia decline within 24-48 hours, while quinine showed slower clearance. For *P. falciparum*, DHA-PPQ achieved rapid near-complete clearance, whereas quinine showed delayed response. Baseline parasite density was higher in *P. falciparum* than *P. malariae*. Anemia was prevalent at baseline (67%). Hemoglobin improved significantly over follow-up (Two-way ANOVA, Time: $F(4,238)=4.51$, $p=0.002$), with no difference between treatments (Treatment: $F(1,238)=1.10$, $p=0.29$). Least-squares mean hemoglobin was 11.17 g/dL (DHA-PPQ) vs. 11.34 g/dL (quinine) (difference: -0.17 g/dL, 95% CI: -0.49 to 0.15). This pilot study demonstrates DHA-PPQ provides superior and more rapid parasite clearance than quinine for both *P. malariae* and *P. falciparum*, with equivalent anemia recovery. These data address a critical evidence gap for non-falciparum malaria and support ACT as first-line treatment for *P. malariae* infections. Larger confirmatory studies are warranted.

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PHARMACOKINETICS OF INTRAVENOUS CIPARGAMIN IN SEVERE *PLASMODIUM FALCIPARUM* MALARIA: RESULTS FROM THE KARISMA STUDY

Vinay Kumar Venishetty[1], Yesenia Franco[2], Helen Gu[2], Ivan Demin[3], Preetam Gandhi[4], Arnab Mukherjee[5]
[1]Novartis Healthcare Private Limited, Hyderabad, India, [2]Novartis Pharmaceutical Corporation, East Hanover, NJ, United States, [3]Novartis Pharma AG, Basel, Switzerland, [4]Novartis International AG, Basel, Switzerland, [5]BioMedical Research, Novartis, Cambridge, MA, United States

Rapid parasite clearance is critical in severe *Plasmodium falciparum* malaria. Cipargamin (KAE609) is a fastacting antimalarial that demonstrated rapid parasite clearance in uncomplicated malaria. The KARISMA study evaluated intravenous (IV) cipargamin to support dose selection and characterize pharmacokinetics (PK) across adult and pediatric patients with severe malaria, including the impact of alpha1acid glycoprotein (AAG). KARISMA was an adaptive, multicenter, randomized, openlabel, sequential cohort study enrolling participants ≥6 months with moderately severe or severe malaria. Adolescents and adults (Cohorts 1–2; ≥12y) received IV cipargamin 20 mg or 40 mg once daily for 2–3 days to establish safety, efficacy, and PK. Dose selection was guided by prior exposure–response relationships (AUC0-24h and parasite reduction ratio) and exposure limits established in earlier studies. Following an interim analysis, exposures achieved with 40 mg in adults/adolescents were targeted to select the doses for pediatric

cohorts (Cohorts 3–5; 6 months–<12y) using different bodyweight bands. In adults, cipargamin exposure increased doseproportionally; a twofold increase from 20 mg to 40 mg resulted in an approximately twofold increase in AUC0-24h and Cmax. After two 40 mg doses, geometric mean AUC0-24h was ~22,000–28,500 ng·h/mL and Cmax ~2,600–3,600 ng/mL, with accumulation of ~1.4–1.8fold. Cipargamin exhibited low clearance (~1 L/h), low volume of distribution (~24 L), and a terminal halflife of ~13–21 h. Pediatric participants (6–<12y) showed ~1.3fold higher exposure with lower clearance and volume of distribution and a shorter halflife (~10 h). Baseline AAG concentrations were higher in severe malaria, particularly in younger children, and increased AAG was associated with higher cipargamin exposure, consistent with enhanced plasma binding and reduced distribution. In conclusion, 40 mg and equivalent body weight band based doses of IV cipargamin provided effective and safe exposures in adult and pediatric patients with severe malaria.

7399

KALUMA PHASE III EXTENSION PHASE - REPORTING SAFETY AND EFFICACY OF REPEAT DOSING OF GANAPLACIDE/LUMEFANTRINE-SDF IN PATIENTS WITH UNCOMPLICATED MALARIA.

Cornelis Winnips[1], Celine Risterucci[2], Issaka Sagara[3], Abdoulaye Djimde[4], Anne Claire Marrast[5]

[1]Novartis, Flueh, Switzerland, [2]Novartis International AG, Basel, Switzerland, [3]University of Bamako (USTTB), Bamako, Mali, [4]MRTC-USTTB, Bamako, Mali, [5]Medicines for Malaria Venture (MMV), Geneva, Switzerland

KALUMA is a randomized, open label Phase III clinical trial (NCT05842954) comprising a core phase and an extension phase. The core phase concluded in 2025 in 12 countries in Sub-Saharan Africa. Of 1614 patients who completed the core phase, 1532 (95%) agreed to participate in the extension phase. The extension phase assessed safety, tolerability, and efficacy of repeated Ganaplacide/Lumefantrine-SD treatment over up to 2 years after completion of the core phase. Patients completing the core phase without early treatment failure or serious adverse events related to study drugs could enter the extension phase, where all malaria episodes were treated with Ganaplacide/Lumefantrine-SDF. Treatment was administered orally once daily for three days under fed conditions, with doses adjusted by body weight. Eligibility for treatment was reassessed at each malaria episode, and rescue medication was allowed if necessary. During the extension phase, 393 patients experienced a total of 495 malaria episodes. Compliance with treatment and meal requirements was high across treatment sequences. Repeated treatment with Ganaplacide/Lumefantrine-SDF maintained high efficacy and an acceptable safety and tolerability profile in adults and children with uncomplicated *P. falciparum* malaria. No new safety signals or cumulative toxicity findings were observed. These results support Ganaplacide/Lumefantrine-SDF as a an effective and well tolerated treatment option, including in settings requiring repeated malaria treatment

7400

FIRST-TRIMESTER ARTEMETHER-LUMEFANTRINE VERSUS QUININE EXPOSURE AND PREGNANCY LOSS: CAUSAL INFERENCE USING PREGNANCY REGISTRY DATA FROM AFRICA

Aninda Nishat Moitry[1], Stephanie Dellicour[2], Philippe J. Guérin[1], Hellen C. Barsosio[3], Halidou Tinto[4], Makoto Saito[1], James A. Watson[1]

[1]Infectious Diseases Data Observatory, Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom, [2]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [3]Kenya Medical Research Institute, Centre for Global Health Research, Kenya, Kisumu, Kenya, [4]Institut de Recherche en Sciences de la Santé, Clinical Research Unit of Nanoro, Nanoro, Burkina Faso

Pregnant women are routinely excluded from clinical trials, leaving critical evidence gaps in first-trimester safety when teratogenic risk is greatest. Although WHO recommended artemether-lumefantrine (AL) for first-trimester use in 2022, continuous efforts are needed to monitor safety data and strengthen evidence. Pregnancy cohorts provide real-world safety data but require explicit causal frameworks for valid inference. We applied causal inference methods within a pregnancy registry from Kenya and Burkina Faso (2021-2025) to assess first-trimester antimalarial safety. We emulated an intention-to-treat target trial comparing AL vs quinine (QN) among pregnancies treated for uncomplicated malaria at 2-14 weeks gestation, with time-zero at treatment initiation. Primary outcome was time to spontaneous pregnancy loss. Directed acyclic graphs and a negative-control analysis among malaria-unexposed pregnancies informed confounder selection. Country-specific Bayesian propensity score models generated stabilised inverse-probability weights (IPW) to create a pseudo-randomised population. We fitted weighted Cox models with left truncation and adjusted for gestational age at pregnancy detection. Of 15,469 pregnancies, 620 were eligible for the hypothetical target trial (310 per country). Treatment allocation differed markedly by setting, with AL predominating in Kenya and QN in Burkina Faso, reflecting distinct temporal and site-specific prescribing patterns. In Kenya, pregnancy loss occurred in 17/255 AL vs 5/55 QN (IPW-weighted hazard ratio 0.43, 95% CI 0.14-1.29). In Burkina Faso, with 1/68 AL vs 11/242 QN (weighted rates: 1.1% vs 3.6%), event sparsity precluded causal modelling. Our findings confirm previous observational meta-analyses suggesting a lower pregnancy loss risk with AL than QN. Robust post-marketing surveillance studies of antimalarials in pregnancy are critical to reduce uncertainty and support informed treatment choice, and a target trial emulation framework and IPW methods applied to these data can generate causally interpretable safety evidence where randomised trials are infeasible.

7401

KARAMA: DOUBLEBLIND, RANDOMIZED, ACTIVE-CONTROL, MULTI-CENTER PHASE IIB/III TRIAL COMPARING THE EFFICACY AND SAFETY OF INTRAVENOUS KIN609VERSUSINTRAVENOUS ARTESUNATE IN SEVERE MALARIA PATIENTS

Preetam Gandhi[1], Evgeniya Reshetnyak[2], Paschal Apanga[3], Bojan Sekeres[1], Vinay Kumar Venishetty[4], Ivan Demin[1], Jared Kenneth Thornton[3], Diogo Mato[1], Chigo Munthali[3]

[1]Novartis Pharma AG, Basel, Switzerland, [2]Novartis Pharmaceuticals Corp, East Hanover, NJ, United States, [3]Novartis Pharmaceuticals UK Ltd, London, United Kingdom, [4]Novartis Healthcare Private Limited, Hyderabad, India

Severe *Plasmodium falciparum* malaria remains a major cause of morbidity and mortality globally, particularly among children under five in sub-Saharan Africa, while recent epidemiological shifts show rising incidence among older children and adults. Intravenous artesunate is the WHO recommended standard of care; however, emerging partial artemisinin resistance threatens its efficacy and there is an urgent need for novel, fast acting, non-artemisinin parenteral therapies. KIN609 is an IV fixed-dose combination of cipargamin, a potent PfATP4 inhibitor active against artemisinin resistant strains, and INE963, a novel non-artemisinin antimalarial with a long elimination half-life. This Phase IIb/III multicentre, double blind, active controlled trial evaluates IV KIN609 followed by oral ACT therapy in patients aged ≥6 months with severe *Plasmodium falciparum* malaria. Approximately 200 participants will be enrolled in Phase IIb and ~800 in Phase III from multiple sites across Sub Saharan Africa. Participants will be randomized 1:1 to receive IV KIN609 or IV artesunate. The primary endpoint is the proportion of participants achieving clinical success by 48 hours (i.e., resolution of prostration, respiratory distress, and altered consciousness before switching to oral therapy) and survival through Day 29. The key secondary endpoint is proportion of participants achieving 99% parasite clearance before switch to oral therapy by 48 hours. This study will generate pivotal evidence on the efficacy, safety, and PK of IV KIN609 compared with IV artesunate. The study outcomes are expected to support the use of KIN609 as a novel, fast acting alternative parenteral therapy for severe *P. falciparum* malaria and to address the growing resistance to current SoC treatments.

7402

AUTOINDUCTION OF CLEARANCE APPEARS TO SHAPE ARTEMETHER PHARMACOKINETICS IN ZAMBIAN CHILDREN WITH UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA

Behnoosh BonakdarYazdi[1], Sandra Massih[2], Jay Sikalima[3], Jean-Bertin Bukasa Kabuya[4], Francesca Aweeka[5], Liusheng Huang[6], Matthew M. Ippolito[1]

[1]Johns Hopkins University School of Medicine, Baltimore, MD, United States, [2]Baltimore, MD, United States, [3]Churches Health Association of Zambia, Ndola, Zambia, [4]Tropical Diseases Research Centre, Ndola, 8, Zambia, [5]San Francisco, CA, United States, [6]UCSF, San Francisco, CA, United States

Artemether-lumefantrine (AL) is first-line therapy for pediatric malaria, yet artemether PK in young children remains poorly defined. We developed a population PK model for artemether in Zambian children 6 months to 5 years old with uncomplicated *Plasmodium falciparum* malaria to characterize disposition and identify determinants of variability. Seventy-five children in a randomized trial received standard oral AL (6 doses over 3 days) with intensive plasma sampling to 72 hours. Artemether concentrations were quantified by LC-MS/MS and analyzed using nonlinear mixed-effects modelling. Structural model development evaluated absorption, disposition, and time-varying clearance; covariate analysis assessed body weight and age. Artemether PK was best described by a 2-compartment model with first-order absorption and time-dependent clearance. Clearance increased across successive doses, consistent with autoinduction, and inclusion of this effect improved model fit and diagnostic performance. Allometric scaling of clearance and intercompartmental clearance to body weight, and volumes scaled to weight, further improved fit. After accounting for size, younger children had lower apparent clearance than older children, supporting age-related maturation of artemether disposition. Artemether in young Zambian children demonstrates 2-compartment disposition with first-order absorption and treatment-related autoinduction of clearance. Body weight and age are important determinants of exposure. This model provides a framework for linking artemether exposure to parasite and gametocyte clearance and for optimizing dosing to improve cure and reduce transmission.

7403

DISTINCT CELLULAR AND HUMORAL IMMUNE PROFILES ELICITED BY ADJUVANTS IN A CLINICAL TRIAL OF THE NA-GST-1 HOOKWORM VACCINE

Camila Queiroz-Glauss[1], Jessica Dade[1], Caroline DiRosato[1], Maria Elena Bottazzi[2], Peter Hotez[2], Jeffrey Bethony[1], David Joseph Diemert[1], Pedro Henrique Gazzinelli-Guimaraes[1]

[1]George Washington University, Washington, DC, United States, [2]Baylor College of Medicine, Houston, TX, United States

Current hookworm control relies on anthelmintic drugs that clear infection but do not prevent reinfection, underscoring the need for an effective vaccine. *Necator americanus* Glutathione-S-Transferase-1 (Na-GST-1) is an advanced vaccine candidate, yet the optimal adjuvant remains undefined. We aimed to compare the immune responses induced by Na-GST-1 formulated with Alhydrogel alone or in combination with CpG. Samples were obtained from vaccinated participants enrolled in a Phase 1 Clinical Trial following a three-dose vaccination schedule. Peripheral blood mononuclear cells were analyzed using multiparameter flow cytometry and unbiased clustering to characterize antigen-specific $CD4^+$ T-cell subsets. Cytokine secretion profiles were assessed by Luminex following in vitro re-stimulation with GST-1. Circulating antibody levels in plasma were accessed by ELISA. CpG-formulated vaccine induced higher GST-1-specific IgG levels, peaking after second dose and remaining sustained throughout the study, but showed limited activation of the T-cell compartment. Alhydrogel formulation elicited inconsistent IgG responses that peaked after each dose but weren't sustained, while promoting expansion of $CD4^+$ T-cell subsets and increased cytokine secretion. GST-1/Alhydrogel-induced $CD4^+$ T cells corresponded to effector memory Th1 subsets defined by CXCR3/CD69/IFN-γ and CD69/CD154/IFN-γ/TNF expression. Luminex analysis demonstrated robust induction of both type 1 (IFN-γ, TNF-α) and type 2 (IL-4/5/13/10) cytokines upon antigen stimulation, particularly after the third dose. Ongoing single-cell and B-cell analyses aim to further understand these responses. These data suggest that Alhydrogel may drive functional remodeling of $CD4^+$ T-cells toward polyfunctional responses, whereas CpG preferentially enhances humoral immunity, highlighting distinct immune compartments engaged by each adjuvant.

7404

ANEMIA RISK AND HEPATIC FIBROSIS IN THE CONTEXT OF *SCHISTOSOMA JAPONICUM* INFECTION

Kirsten d'Hemecourt[1], Mario Jiz[2], Hannah Wu[3], Susannah Colt[4], Ralph Narido Aniceto[5], Joachim Richter[6], Jennifer F. Friedman[7]

[1]Brown University Health, Providence, RI, United States, [2]Research Institute for Tropical Medicine, Muntinlupa City, Philippines, [3]Brown University, School of Medicine, Lincoln, RI, United States, [4]Rhode Island Hospital, Providence, RI, United States, [5]Research Institute for Tropical Medicine, Tacloban, Philippines, [6]Univ of Duesseldorf, Duesseldorf, Germany, [7]Center for International Health Research, Providence, RI, United States

Schistosomiasis affects over 200 million people worldwide, with significant morbidity driven by chronic infection. Complications of intestinal *Schistosoma japonicum* infection include anemia and hepatic fibrosis, yet the relationship between these key morbidities is poorly understood. Anemia may result from iron loss through intestinal bleeding or from chronic inflammation related to immune responses to schistosome eggs and adult worms. The extent to which hepatic fibrosis and related inflammation contribute to anemia remains unclear. These baseline findings of a longitudinal cohort study seek to characterize the prevalence and type of anemia and to assess how anemia relates to hepatic fibrosis in individuals with *S. japonicum* infection recruited from three villages in Leyte, The Philippines. Baseline liver ultrasounds, CBC, ferritin, and IL-6 levels were collected. Iron deficiency anemia (IDA) was defined as low hemoglobin for age and sex and ferritin ≤ 30 ng/mL using WHO cut-offs. Non-iron deficiency anemia (NIDA) was defined as low hemoglobin for age and sex and ferritin > 30 ng/mL. Hepatic fibrosis is assessed and graded using standardized ultrasonography based on the WHO Basel protocol. Among 369 participants, the prevalence of anemia was 10.0%. Iron deficiency was present in 39.3% of participants, while IDA and NIDA were present in 4.6% and 5.4%, respectively. The highest anemia prevalence appears in the those with interseptal (12.2%) and combined portal and interseptal (11.8%) fibrosis patterns. In the multivariate model, interseptal fibrosis was associated with significantly higher odds of iron deficiency anemia (OR 4.72, p=0.02). Male sex was strongly associated with non-iron deficiency anemia (OR 4.82, p=0.04), while men had lower odds of iron deficiency anemia (OR 0.20, p=0.003). Fibrosis pattern was not significantly associated with overall anemia prevalence. Participants with NIDA have the highest proportion of detectable IL-6 (25%) compared to IDA (11.8%) and no anemia (13.3%) but this association was not significant, $X^2(2, N=369) = 2.25$, $p = 0.325$.

7405

MAPPING THE EXPOSOME OF ZIMBABWEAN PRESCHOOL-AGED CHILDREN (PSAC) USING A NOVEL PEPTIDE MICROARRAY APPROACH

Emilee J. Benos

The University of Edinburgh, Edinburgh, United Kingdom

Collective prior exposure to non-pathogenic immunogens and infectious agents, including immunomodulatory helminths and anthelminthic treatment, shapes host future immune responses to related and unrelated immunogens. With the widespread rollout of mass drug administration (MDA) across Africa, many populations have now received at least one round of deworming treatment. This raises critical questions about the nature, persistence, and significance of immunological shifts in endemic settings with repeated exposures. Thus, the aim of this proof-of-concept study was to survey and define the exposome, or the collection of

immunogens a host has been exposed to, of a helminth-endemic rural Zimbabwean population. Using a bespoke peptide microarray, we determined antigen-specific serum IgM and IgG reactivities of 20 children under five years of age against a variety of B-cell epitopes from 290 immunogens of interest to Zimbabwe, including pathogens, vaccines, allergens, autoantigens, and a variety of parasites. Data from this microarray revealed broad, heterogenous immunogen recognition early in life. The most frequently recognized exposome constituted viruses, bacteria, fungi, autoantigens, and both micro- and macro-parasites, including helminths, trypanosomes, and plasmodium species. This diverse early-life infectome signature may contribute to differential susceptibility to infection severity, vaccine responsiveness, or immune-mediated pathology. Results from the present discovery study provide baseline proof of concept reference values for helminth-endemic under five immune ecologies and should be considered in future public health planning.

7406

BEYOND EOSINOPHILIA: HEMATOLOGIC ALTERATIONS ASSOCIATED WITH *SCHISTOSOMA JAPONICUM* AND SOIL-TRANSMITTED HELMINTH POLYPARASITISM IN A SCHISTOSOMIASIS-ENDEMIC COMMUNITY IN LEYTE, PHILIPPINES: A RETROSPECTIVE CROSS-SECTIONAL STUDY

Ralph Narido Aniceto, Mario Antonio Jiz, II
Research Institute for Tropical Medicine, Tacloban, Philippines

Helminth polyparasitism remains common in schistosomiasis-endemic regions, yet its broader hematologic consequences beyond eosinophilia are not well characterized. In many endemic settings, *Schistosoma japonicum* and soil-transmitted helminths (STH) frequently co-occur due to shared environmental and behavioral risk factors. While eosinophilia is a recognized hallmark of helminth infection, other hematologic alterations associated with concurrent infections remain less clearly defined. This study examined non-eosinophil hematologic profiles among individuals with single helminth infection and polyparasitism in a schistosomiasis-endemic community in Leyte, Philippines. We conducted a retrospective cross-sectional analysis of parasitologic and hematologic records from 420 infected individuals aged 14-60 years. Infection status was determined using Kato-Katz stool microscopy, and participants were classified as having single infection (n = 175) or polyparasitism (n = 245), defined as *S. japonicum* infection with at least one STH species. Hematologic parameters were obtained from complete blood counts, excluding eosinophil counts. Co-infection with STH was common, with hookworm detected in 48.3% of participants, followed by *Ascaris lumbricoides* (20.5%) and *Trichuris trichiura* (19.1%). Most *S. japonicum* infections were of light intensity (89.1%), with a mean intensity of 52.19 ± 161.10 eggs per gram. Significant differences in red blood cell indices were observed. Mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) were associated with parasitic infection status ($p = 0.020$ and $p = 0.035$, respectively), while hemoglobin, platelet count, red blood cell count, and total leukocyte count did not differ significantly. Helminth polyparasitism was associated with subtle but measurable alterations in red blood cell indices independent of eosinophilia, suggesting broader subclinical hematologic effects. Incorporating routine hematologic indicators into surveillance may improve detection of hidden morbidity and strengthen parasite control strategies in endemic settings.

7407

TRICHURIS TRICHIURA INFECTION DRIVES AN EXPANSION OF TH2-ASSOCIATED CD4+ T CELLS

George Sellers, Harris Droghini, Thomas B. Nutman
National Institutes of Health, Bethesda, MD, United States

Despite the high worldwide prevalence of infection with *Trichuris trichiura* (Tt), the human whipworm, little is known about the systemic immune response driven by Tt infection. Moreover, given that Tt has been shown to have potential as a therapeutic for ulcerative colitis through the induction of CD4+IL22+ cells in the intestine based on a single human case report, understanding the nature of the T cell response in Tt infection is of paramount importance. To provide insight into the human T cell responses to *Tt* infection, we used a T cell focused multiparameter flow cytometry panel to assess the response of peripheral blood mononuclear cells (PBMCs) in 16 subjects with proven Tt infection (many with coincident loiasis) and 7 uninfected healthy volunteer (HV) subjects. We compared baseline conditions and exposure to optimal concentrations of *Trichuris muris* excretory/secretory (Tm ES) antigen, soluble filarial *Brugia malayi* antigen (BMA), CEFX (a multi viral peptide pool), soluble *Candida albicans* extract, and Staphylococcus enterococcus B (SEB) superantigen. At baseline, there were no differences in numbers of CD3+, CD4+, and CD8+ cells, however we observed an 138% increase of the CRTH2+ effector memory CD4+ T cells (Th2) population in the Tt-infected patients compared to healthy controls, suggesting that the Tt infection drove an expansion of Th2-associated CD4+ cells. Although there were few differences seen in any parameter between the Tt-infected subjects and the HVs in response to CEFX, Candida, or SEB, interestingly, TmES induced an increase in CXCR5 in effector memory cells ($p=0.00627$), central memory cells ($p=0.0114$), and Treg cells ($p=0.0246$) in patients compared to HVs suggesting that Tt infection and their secreted antigens may induce a shift towards follicular homing by expanding early T follicular helper-like cells. We are profiling the cytokines secreted in the culture supernatants evaluating these cell populations of interest through transcriptional analyses. Furthermore, the ontogeny of the immune response in Tt infection will be assessed in an ongoing *T. trichiura* controlled human infection model.

7408

MONOCYTE-ASTROCYTE CROSSTALK INDUCES LEUKOCYTE RECRUITMENT IN NEUROCYSTICERCOSIS THROUGH CHEMOKINE NETWORKS

Luz M. Toribio[1], Deborah L. Chong[2], Hector H. Garcia[1], Jon Friedland[2]
[1]Universidad Peruana Cayetano Heredia, Lima, Peru, [2]City St.George's University of London, London, United Kingdom

Neuroinflammation caused by neurocysticercosis (NCC), a neglected parasitic brain infection and a leading cause of acquired epilepsy globally, is driven by the host inflammatory response when cysts degenerate. Monocytes respond directly to parasite antigens, but whether they induce secondary chemokine responses in astrocytes, major contributors of neuroinflammation, remains unclear. Furthermore, the astrocytes chemokine repertoire remains ill-defined. We investigated whether monocyte-derived signals drive astrocyte chemokine production when exposed to conditioned media from monocytes stimulated with *Taenia solium* total antigen extract (Tsol). Human monocytes from healthy donors were stimulated with Tsol for 24, 48 and 72 h. Then, monocyte-conditioned media collected after 24 h of stimulation (CoMTsol) was applied to a primary culture of human astrocytes (HA). Chemokine secretion and gene expression were assessed for monocytes and HA at 8, 24, 48 and 72 h and chemokine levels of CXCL1, CXCL8, CXCL10 and CCL2 were measured by ELISA and RT-qPCR. Our results showed that Tsol-stimulated monocytes only produced increased secretion of CXCL8 from 24 h onwards (2122.44 pg/ml), as well as significant upregulation of gene expression ($p=0.0022$ compared to controls). On the other hand, astrocytes exposed to CoMTsol exhibited significant secretion of all four chemokines, this response was time-dependent, significantly increasing at 24 h (all $p<0.022$) and sustained and peaking at 72 h (all $p<0.0081$) post-stimulation (CXCL1=4388.7 pg/ml, CXCL8=6233.21 pg/ml, CXCL10=5521.8 pg/ml and CCL2=18930.55 pg/ml; all at 72 h) compared to unstimulated HA. Gene expression was consistent with secretion data showing significant upregulation of genes in HA stimulated with CoMTsol ($p<0.01$). These findings demonstrate that monocyte-astrocyte crosstalk induces chemokine responses associated with recruitment of monocytes, neutrophils, and T cells, suggesting that astrocytes are a key source of these, which may contribute to leukocyte recruitment and amplification of neuroinflammation in NCC.

7409

A NOVEL ANTHELMINTIC CRYSTAL PROTEIN

Kelly A. Flanagan[1], Hanchen Li[2], Qian Ding[2], Florentina Rus[3], Duy Hoang[1], Stefani Diaz Valerio[4], Gary Ostroff[1], Heiko Liesegang[4], Raffi V. Aroian[1]

[1]UMASS Medical School, Worcester, MA, United States, [2]Worcester, MA, United States, [3]Florentina Rus, Worcester, MA, United States, [4]Georg-August-University, Göttingen, Germany

Gastrointestinal nematodes (GINs) are parasitic nematodes that cause significant morbidity among livestock and impoverished humans worldwide and globally exhibit increasing resistance to currently approved therapies. Several anthelmintic Cry proteins from *Bacillus thuringiensis* (Bt) are differentially bioactive against GINs and are demonstrably curative therapies for GIN infections, presenting an opportunity to meet the urgent need for new drugs with unique modes of action against GINs. In our efforts to increase the repertoire of nematicidal Cry proteins, we used published IDOPs methods and ex vivo assays with GINS to further identify novel Cry proteins with anthelmintic capabilities, one of which we present here. This novel nematicidal Cry protein, tentatively named CryH1, is recombinantly expressed, quantitated, and assayed against various nematodes. Our results indicate that CryH1 exhibits potent bioactivity against the GINs *Ancylostoma ceylanicum* hookworms and *Ascaris suum* roundworms, both ex vivo and in vivo, and *Trichuris muris* whipworms ex vivo. Surprisingly, CryH1 has no apparent toxic effects on the free-living nematode *Ceanorhabditis elegans*. We further screen for and detect synergistic bioactivity between this and other published nematicidal Cry proteins, potentially demonstrating the ability to lower the total Cry dose toward the target product profile of Cry proteins for therapeutic interventions while potentially delaying resistance via Cry combinations.

7410

ASSESSING THE PREVALENCE AND GENETIC CHARACTERISTICS OF *STRONGYLOIDES SPECIES*IN SOUTH-EASTERN GHANA

Grace Opoku Gyamfi[1], Yvonne Ashong[1], Millicent Opoku[2], Millicent Selassie Afatodzie[1], kwasi Agyenkwa-Mawuli[1], Emmanuel Gyekye Frimpong[1], Seth Offei Addo[1], Daniel Oduro[3], Dziedzom Komi de Souza[1]

[1]Noguchi Memorial Institute for Medical Research, Legon, Ghana, [2]La Trobe University, Melbourne-Victoria, Australia, [3]University Of Ghana, Legon, Ghana

Strongyloidiasis is a parasitic disease caused by the facultative nematode *Strongyloides stercoralis,* with important but often underrecognized public health implications. In Africa, the disease remains understudied, particularly in relation to prevalence and genetic diversity, largely due to the low sensitivity of conventional diagnostic tools and limited surveillances systems. This study analysed 1392 archived human stool samples collected from seven communities across three districts in South-eastern Ghana. DNA extraction and PCR followed a pooling strategy (with up to 10 stool samples per pools). Positive pools were subsequently unpooled and tested individually for confirmation. The study reported an overall pooled infection rate of 7.2% (95% CI: 5.5-9.3) and an individual infection rate of 18.5% (95% CI: 16.5-20.6). Logistic regression analysis showed no significant associations between infection status and demographic factors such as sex, age, or district. Of 40 PCR positive samples that were Sanger sequenced 17 were of good enough quality for analysis. Phylogenetic analysis based on 18S rRNA sequences indicated that most Ghanaian isolates clustered together with a few groupings alongside reference sequences from Tanzania and Australia. The haplotype network population showed moderate nucleotide diversity ($\pi = 0.2427$) with several segregating sites (S=53), representing minor genetic variations, suggesting a genetically homogeneous Ghanaian *S. stercoralis* population with limited intraspecific variation. This study provides evidence of *Strongyloides spp*., prevalence and genetic relatedness in Southeastern Ghana. The findings highlight the need for more sensitive diagnostic approaches and strengthened, integrated surveillance to support targeted control strategies and public health interventions.

7411

METAGENOMIC INSIGHTS INTO DRUG RESPONSE TO ALBENDAZOLE AND ALBENDAZOLE-IVERMECTIN COMBINATION THERAPY IN SOIL-TRANSMITTED HELMINTH INFECTIONS

Javier Gandasegui[1], Pedro Fleittas[2], Yan Shao[1], Augusto Messa Junior[3], Woyneshet Gelaye[4], Valdemiro Novela[3], Auria De Jesus[3], Dereje Bedane[4], Wendemagen Enbiale[4], Inacio Mandomando[3], Lisette Van Lieshout[5], Alejandro J. Krolewiecki[6], Trevor Lawley[1], Jose Muñoz[2], Doyle Stephen R.[1]

[1]Wellcome Sanger Institute, Saffron Walden, United Kingdom, [2]Barcelona Institute for Global Health, Barcelona, Spain, [3]Centro de Investigação em Saúde de Manhiça, Manhiça, Mozambique, [4]Bahir Dar University, Bahir Dar, Ethiopia, [5]Leiden University Medical Center, Leiden, Netherlands, [6]Universidad Nacional de Salta, Oran, Argentina

Soil-transmitted helminths (STH) remain a major cause of morbidity in endemic regions, yet little is known about how anthelmintic treatment and the gut microbiome interact and influence therapeutic outcomes. The ALIVE clinical trial compared the current standard of care for STH infections, the albendazole, against a new fixed-dose coformulation (FDC) of albendazole plus ivermectin. To investigate the role of the gut microbiome in the context of treatment response, we performed shotgun metagenomic sequencing on paired pre- and post-treatment stool samples from 429 participants from Ethiopia and Mozambique. After quality control, microbial taxonomic profiling was conducted using Sylph. Diversity metrics were computed as Shannon index and Bray-Curtis distance, and differential-abundance testing was carried out combining outputs from MaAsLin3 and ANCOM-BC. We have evaluated the association between the gut microbiome composition with baseline variables, such as country of origin, age, sex, infection intensity, etc; we have assessed if the gut microbiome can influence the safety or efficacy; and we have also evaluated the impact of the different drug regimens in the gut microbiome composition. Analyses revealed marked geographic differentiation in gut microbiome composition between Ethiopian and Mozambican participants. Despite this ecological divergence, no consistent microbiome signatures were associated with treatment efficacy or safety outcomes across arms. However, a robust post-treatment decrease in Enterobacteriaceae was observed when children were treated with the FDC, suggesting a conserved response of this bacterial family to anthelmintic exposure or to infection clearance. Current work focuses on generating metagenome assembled genomes for functional annotation and resistome profiling to identify microbial pathways and antimicrobial-resistance genes that may modulate drug bioavailability, metabolism, or tolerability. These findings could provide a foundation for future microbiome-aware strategies in STH control in endemic countries.

7412

IMPACT OF DTG- LPV/R-, AND EFV-BASED ANTIRETROVIRALS ON PIPERAQUINE PHARMACOKINETICS AND CARDIAC SAFETY IN UGANDAN CHILDREN LIVING WITH HIV

Norah Mwebaza[1], Richard Kajubi[1], Malik Koire[2], Meghan Whalen[3], Florence Marzan[3], Sindy He[3], Justin Goodwin[4], Kacey Richards[5], Bishop Opira[1], Moses Mwima[1], Grace Kisitu[2], Rogers Sekabira[2], Adeodata Kekitiinwa[2], Moses R. Kamya[1], Francesca Aweeka[3], Sunil Parikh[5], **Liusheng Huang**[3]

[1]Infectious Diseases Research Collaboration, Kampala, Uganda, [2]Baylor College of Medicine, Kampala, Uganda, [3]School of Pharmacy, University of California San Francisco, San Francisco, CA, United States, [4]Yale University School of Medicine, New Haven, CT, United States, [5]Yale University School of Public Health, New Haven, CT, United States

Dihydroartemisinin-piperaquine (DHA-PPQ), a widely used artemisinin-based combination therapy for malaria treatment and prevention, is often

co-administered with antiretrovirals (ARV) in children living with HIV (CLHIV) in malaria endemic region such as Uganda. Dolutegravir (DTG)-, lopinavir/ritonavir (LPV/r)-, and efavirenz (EFV)-based ARV, which are commonly used in children, share metabolism pathways with DHA-PPQ, namely cytochrome P450 enzymes and drug transporters, raising concerns about potential drug-drug interactions. However, studies of these interactions in children living in co-endemic areas are lacking. We conducted a prospective open-label pharmacokinetics (PK) study of DHA-PPQ in Ugandan CLHIV receiving DTG-, LPV/r- and EFV-based ARV regimens (n=30 each). Children on DTG were 11-17 years old and those on LPV/r or EFV were 3-10 years old, and age-appropriate control children without HIV were enrolled. All children were without malaria. PK sampling was performed over 42 days with PPQ quantification by LC tandem MS. Compared to controls, total PPQ exposure in CLHIV on DTG, as measured by area under concentration-time curve from the 3rd DHA-PPQ dose to day 42, was similar but day 42 PPQ concentrations were reduced by 64% ($p<0.0001$). In contrast, overall PPQ exposure in the LPV/r group increased by 240% ($p<0.0001$), with day 42 PPQ increasing by 91% ($p<0.0001$), while exposure with EFV was reduced by 69% ($p<0.0001$), with day 42 PPQ reducing by 92% ($p<0.0001$). Changes in ECG QTcF intervals were not significant except for two cases: one child on LPV/r reached 458 msec 4 hours post-dose on Day 2 and a child without HIV at day 7 had a >60 msec higher QTcF interval than at baseline. A statistically significant correlation was not found between changes in QTcF interval and PPQ peak concentrations. In conclusion, CLHIV on EFV have significantly reduced PPQ exposure, which may impact efficacy, while those on LPV/r have significantly elevated PPQ exposure, as compared to controls. No significant cardiac toxicity was observed in any cohort. For DTG, lower terminal PPQ exposure may shorten the duration of post-treatment chemoprophylaxis.

7413

PROGRESS TOWARDS ENDING THE TB/HIV SYNDEMIC IN U.S. PRESIDENT'S EMERGENCY PLAN FOR AIDS RELIEF (PEPFAR)-SUPPORTED COUNTRIES, 2020-2024

Brigette L. Gleason[1], Stephanie O'Connor[1], Sevim Ahmedov[2], Michael Reid[2], Macarthur Charles[1], Matthew Brown[2], Anand Date[1]

[1]U.S. Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Bureau of Global Health Security and Diplomacy, U.S. Department of State, Washington, D.C., DC, United States

Tuberculosis (TB) remains the leading cause of death among people living with HIV (PLHIV). About half of TB cases are asymptomatic, complicating case-finding efforts. The U.S. President's Emergency Plan for AIDS Relief (PEPFAR) prioritized integrated TB/HIV care to prevent, detect, and treat TB among PLHIV, and test and treat HIV in people with TB. Using a multiagency approach to support and monitor implementation, PEPFAR saved over 25 million lives. We aimed to assess trends for TB/HIV service delivery in PEPFAR-supported countries. We analyzed programmatic indicators spanning the TB/HIV care cascade during fiscal years (FY) 2020–2024 across 35 countries. PEPFAR-supported sites provided TB screening to 90% of PLHIV on antiretroviral therapy (ART) in FY24, up from 78% in FY20. The number of rapid molecular diagnostic tests—which are also capable of detecting rifampin-resistant TB—more than tripled from 313,790 in FY20 to 1,019,634 in FY24. PEPFAR linked 845,943 PLHIV to TB treatment during FY20–24, averaging 464 per day. Since FY20, 14.3 million PLHIV (including 588,589 children) received TB preventive treatment, known to reduce morbidity and mortality. The percentage of countries that achieved the target of ≥95% of people with TB knowing their HIV status increased from 72% in FY20 to 89% in FY24. Of the 60,040 people with TB who tested positive for HIV in FY24, 96% initiated ART. This analysis highlights marked progress across TB/HIV indicators reported through PEPFAR, but it does not reflect significant TB case-finding gaps resulting, in part, from use of symptom-based screening tools—the predominant approach in high-burden, resource-constrained settings. As countries scale-up adoption of symptom-agnostic screening tools, like chest radiography and rapid molecular testing, along with other recommended strategies, adapting monitoring indicators may help assess impact and guide program planning and resource allocation to advance progress towards TB elimination. PEPFAR's approach to integrated, data-driven service delivery and monitoring can inform countries as they transition to greater self-reliance of TB/HIV programming.

7414

RESOLVING DIAGNOSTIC AMBIGUITY IN SUSPECTED MPOX CASES THROUGH METAGENOMIC SEQUENCING: DETECTION OF CLADE 1B AND INCIDENTAL HIV DRUG RESISTANCE

Stephen Aricha[1], Abdi Roba[1], Jully Awino Okonji[2], Edwin Ochieng[3]

[1]1.Division of National Laboratory Services, National Public Health Laboratory, Nairobi, Kenya, [2]Nairobi, Kenya, [3]Association of Public Health Laboratories, Nairobi, Kenya

Mpox remains a major public health threat in sub-Saharan Africa, with the emergence of Clade 1b underscoring the need for enhanced genomic surveillance. In clinical settings, suspected cases are often difficult to diagnose because symptoms overlap with other febrile and ulcerative illnesses. Rapid laboratory characterization is therefore critical for outbreak control and identification of co-infections that may alter management, particularly in immunocompromised patients. This study evaluated the utility of metagenomic next-generation sequencing (mNGS) in resolving diagnostic uncertainty in suspected mpox cases while characterizing viral lineages and clinically relevant genomic markers. Three clinical samples collected from suspected mpox cases on 17 July 2025 were processed at the National Genomics and Molecular Surveillance Laboratory. Total nucleic acids were extracted using the QIAGEN RNA/DNA kit, followed by cDNA synthesis and library preparation with the Illumina Viral Surveillance Panel v2. Sequencing was performed on an Illumina platform. Bioinformatic analysis used the Illumina DRAGEN Viral Surveillance pipeline, complemented by the CZ ID metagenomic pipeline, with final clade assignment confirmed using Nextclade. mNGS resolved the clinical status of all three cases. One sample was confirmed as mpox with high genomic coverage and classified as Clade 1b. The remaining two were mpox-negative; however, mNGS identified HIV-1 infections and an additional co-pathogen, human papillomavirus. Importantly, one HIV-positive sample harbored reverse transcriptase mutations associated with resistance to nucleoside reverse transcriptase inhibitors while remaining susceptible to non-nucleoside reverse transcriptase inhibitors. DRAGEN-accelerated mNGS proved instrumental in resolving diagnostically complex suspected mpox cases. By simultaneously identifying Clade 1b and detecting actionable HIV drug resistance mutations in mpox-negative patients, this integrated genomic surveillance approach provides both outbreak intelligence and precision clinical insights for resource-limited settings.

7415

CHARACTERIZATION OF EPIDEMIOLOGY AND CARE OUTCOMES OF HIV IN GEORGIA AMIDST ONGOING REGIONAL CONFLICT

Brennan R. Cebula[1], Revaz Metchurtchlishvili[2], Shilpa Hakre[3], Thomas A. Musich[4], Trevor A. Crowell[3], Julie A. Ake[1], Otar Chokoshvili[2], Natia Dvali[2], Pati Gabunia[2], Lana Gatserelia[2], Marine Karchava[2], Anna Nanava[4], Nino Rukhadze[2], Eric R. Schuler[3], Glenna Schluck[3], Lali Sharvadze[2], Roger Ying[3], Akaki Abutidze[2]

[1]U.S. Military HIV Research Program, CIDR, Walter Reed Army Institute of Research, Silver Spring, MD, United States, [2]Tengiz Tsertsvadze Infectious Diseases, AIDS and Clinical Immunology Research Center, Tbilisi, Georgia, [3]Henry M. Jackson Foundation for the Advancement of Military Medicine, Bethesda, MD, United States, [4]Walter Reed Army Institute of Research Europe-Middle East, Tbilisi, Georgia

The HIV epidemics in Russia and Ukraine are the fastest growing in Europe. Conflict in Ukraine has led to significant population displacement in Eastern Europe impacting regional HIV epidemiology, the implications of which on Georgia's HIV epidemiology remain unclear. This cross-sectional study aims to characterize HIV risks, molecular epidemiology, and associated infections among people living with HIV (PLH) receiving care at the Tengiz Tsertsvadze

Infectious Diseases, AIDS and Clinical Immunology Research Center in Tbilisi, Georgia. We are enrolling up to 650 PLH aged ≥18 years into four groups: 1) from Georgia taking antiretroviral therapy (ART); 2) ART-naive; 3) from Abkhazia (Russian-occupied Georgian territory); 4) from non-Georgian Eastern European countries. Data collection includes demographic, health, and sociobehavioral factors, HIV viral load (VL) with genotype/resistance testing for those with VL ≥1000 copies/ml, CD4 count, and associated infection testing. As of February 2026, data for 568 participants were available (median age 39, interquartile range 31-49; 22.4% female). Viral suppression (VL ≤1000 copies/ml) was high in participants from Georgia taking ART (93.9%), Abkhazia (82.1%), and non-Georgian Eastern-European countries (93.1%). Among 120 participants with genotype/resistance testing, subtypes A6 (89.2%) B (7.5%), CRF02_AG/B (2.5%), and F (0.8%) were identified. Drug resistance mutations were detected in 91.6%. Integrase strand transfer inhibitor mutations were present in 94.5% of which 98.1% were the L74I polymorphism. High viral suppression rates suggest effective HIV care in Georgia. Subtype A6 predominance with minor representation of other subtypes reflects regional epidemic influence. L74I is a characteristic subtype A6 polymorphism associated with risk of virologic failure with long-acting cabotegravir. Whether L74I drives this risk, and the implications for cabotegravir use in the region are unclear. Continued data collection and analysis from this study will further clarify HIV and related infection epidemiology in Georgia to inform regional public health strategies.

7416

CHARACTERIZING CAUSES OF DEATH AMONG PEOPLE LIVING WITH HIV IN THE ERA OF WIDESPREAD ART COVERAGE IN SUBSAHARAN AFRICA

Kitiezo Aggrey Igunza[1], Kyu Han Lee[2], Broline A. Sagini[1], Fredrick O. Onduru[1], Edwin Kiplelgo[1], Christopher A. Mugah[1], Hellen Muttai[3]

[1]Kenya Medical Research Institute, Kisumu, Kenya, [2]The Task Force for Global Health, Boston, MA, United States, [3]Liverpool School of Tropical Medicine, Kisumu, Kenya

AIDS deaths in sub-Saharan Africa have fallen ~50–55% since 2010, yet ~380,000 occur annually, with limited capacity for accurate cause-of-death determination. In a crosssectional study in Kenya and Sierra Leone, an expert panel reviewed clinical records, verbal autopsy, and postmortem laboratory results from minimally invasive tissue sampling to assign causes of death. We compared causes of mortality and pathogen profiles by HIV status from adult decedents (2023-2024). Of 622 decedents (55.6% female, median age 55), 154 (24.6%) were HIV-positive. Among HIV-positive decedents compared to HIV-negative, *Mycobacterium tuberculosis* was the most common pathogen (35.6% vs. 17.8%, p=0.002). Opportunistic pathogens, *Toxoplasma gondii* (4.6% vs. 0%, p=0.013), Cryptococcus neoformans (3.4% vs. 0%, p=0.038), Cytomegalovirus (3.4% vs. 0.6%, p=0.12), and Pneumocystis jirovecii (2.3% vs. 0%, p=0.11), were detected almost exclusively in HIV-positive decedents. *Streptococcus pneumoniae* was more frequent in HIV-positive decedents (17.2% vs. 10.7%, p=0.2), while *Escherichia coli* (22.5% vs. 8.0%, p=0.005) and *Klebsiella pneumoniae* (25.4% vs. 17.2%, p=0.2) were more common among HIV-negative decedents. Among HIV-positive decedents, sepsis/bacteraemia (27.0% vs. 19.0%, p=0.045), anaemia (10.6% vs. 3.2%, p=0.001), and meningitis/encephalitis (4.3% vs. 1.3%, p=0.037) were significantly more common compared to HIV-negative decedents. In contrast, heart disease (20.1% vs. 8.5%, p<0.001) and cerebrovascular disease (14.5% vs. 4.3%, p<0.001) were more frequent among HIV-negative decedents. Lower respiratory infections (17.7% vs. 17.1%, p=0.9), cancer (9.8% vs. 7.8%, p=0.5), and liver disease (4.3% vs. 5.0%, p=0.8) were similarly distributed across both groups with no significant differences. HIV-positive patients in SSA still die from preventable opportunistic infections. Prevention, early diagnosis, and prompt treatment of opportunistic infections, particularly tuberculosis, fungal diseases, and sepsis, remain critical to reducing preventable deaths among HIV-positive adults in sub-Saharan Africa. 1

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SELF-SAMPLING FOR HPV DETECTION: AN ACCEPTABLE AND PRACTICAL APPROACH TO CERVICAL CANCER SCREENING AMONG WOMEN LIVING WITH HIV (WLHIV)

Jaya Chakravarty Tapadar, Urvashi Geeta Rai

Banaras Hindu University, Varanasi, Uttar Pradesh, India

Cervical cancer remains a major public health concern in India, with an estimated 123,907 new cases and 77,348 deaths annually, contributing nearly one-fifth of the global burden (WHO, 2024). Persistent infection with high-risk human papillomavirus (HPV), particularly types 16 and 18, is the primary etiological factor. Women living with HIV (WLHIV) are at significantly increased risk of cervical cancer. However, screening coverage in this population remains suboptimal due to barriers related to access, cultural acceptability, and discomfort. Self-sampling for HPV testing represents a reliable, private, and convenient alternative that may improve screening uptake, especially in low-resource and culturally sensitive settings. Between 4 April and 16 May 2025, 675 WLHIV attending an antiretroviral therapy centre were approached, of whom 598 (88.6%) consented to self-collection using COPAN FLOQ swabs. All collected samples were successfully processed and tested for high-risk HPV using the Abbott m2000 system. Among the 598 participants, 162 tested positive for high-risk HPV, yielding a prevalence of 27.1%. The mean age of HPV-positive women was 41.36 ± 9.95 years. Of these, 26 (16.0%) had isolated HPV 16 infection, 4 (2.5%) had HPV 18 alone, and the majority, 99 (61.1%), were infected with other high-risk HPV types (including HPV 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68). Mixed infections were observed in 33 women: 19 (11.7%) with HPV 16 plus other types, 9 (5.6%) with HPV 18 plus others, 2 (1.2%) with both HPV 16 and 18, and 3 (1.9%) with multiple high-risk categories. Among the 78 women who declined participation, the main reasons were prior hysterectomy (n=41), menstruation (n=25), fear of testing (n=7), and pregnancy (n=5). Self-collected HPV sampling demonstrated high acceptability, with an uptake of 88.6% among WLHIV. A substantial burden of high-risk HPV infection was observed, with a predominance of non-HPV 16/18 genotypes. These findings underscore the need to strengthen early HPV detection strategies and support the integration of self-sampling approaches into cervical cancer screening programs for WLHIV.

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INTEGRATED EYE SCREENING IN HIV CLINICS REVEALS UNMET OPHTHALMIC NEED IN MOSHI TANZANIA

Arthi Vaidyanathan[1], Alice Haystead[2], Medrine L. Kihanga[3], Lisa Wanda[4], Kelvin F. Haukila[4], Godfrey L. Kweka[4], Jerome J. Mlangi[4], Tumsifu G. Tarimo[4], Pankrasi S. Shayo[4], Blandina T. Mmbaga[4], Holly Rawizza[3], Einoti Y. Matayan[5], Preeti Manalavan[6], Nathan M. Thielman[7], Sharon Fekrat[2], Leon W. Herndon[2], Lloyd Williams[2], Julian Hertz[7]

[1]Duke University School of Medicine, Durham, NC, United States, [2]Department of Ophthalmology, Duke Eye Center, Durham, NC, United States, [3]Division of Infectious Diseases, Brigham and Women's Hospital, Boston, MA, United States, [4]Kilimanjaro Clinical Research Institute, Moshi, United Republic of Tanzania, [5]Department of Ophthalmology, Kilimanjaro Christian Medical Center (KCMC), Moshi, United Republic of Tanzania, [6]Division of Infectious Diseases and Global Medicine, Department of Medicine, University of Florida, Gainesville, FL, United States, [7]Duke Global Health Institute, Durham, NC, United States

With improved treatment and life-expectancy, people living with HIV (PLWH) in sub-Saharan Africa face a growing burden of non-infectious ocular disease that remains understudied and unaddressed. We hypothesized that clinic-based ophthalmic screening would identify a substantial prevalence of unrecognized ocular disease among PLWH. We conducted a cross-sectional eye screening study among PLWH ≥40 years within 3 HIV clinics in Moshi, Tanzania from September 2025 to March 2026. Participants were referred for ophthalmic evalutaion if they had moderate or severely bothersome self-reported symptoms, pinhole visual acuity (VA) <20/60, intraocular pressure (IOP) >23 mmHg, visual field deficit on confrontation testing, or fundus abnormalities on remote ophthalmologist

review. Referred participants were contacted via telephone after 1 month to assess outcomes. Of 175 PLWH enrolled (75% female; mean age 53 years; median HIV duration 9 years; 95% virally suppressed), most (126, 72%) had never received eye care. Overall, 54 (31%) participants met one or more referral criteria, for fundus abnormalities (31, 18%), symptoms (25, 14%), VA (13, 7%), visual field (9, 5%), and IOP (1, 0.6%). On multivariable analysis, age was associated with referral (aOR 1.07/ year, 95% CI 1.02–1.12; p=0.002), while HIV duration, current CD4, and viral load did not reach significance. Only 11/54 (20%) referred participants completed ophthalmic evaluation within 1 month. Among those, 10/11 (91%) received actionable diagnoses including refractive error, retinal pathology, glaucoma, and surgical indications. Self-reported barriers among the 22 participants reached that had not completed evaluation included cost 12 (55%), competing health priorities 6 (27%), lack of time 5 (23%), and travel constraints 3 (14%). HIV clinic-based eye screening identified a high burden of ophthalmic need among PLWH, but gaps in linkage to care limit clinical benefit. Larger studies are needed to define disease burden and inform screening recommendations, including integration of eye care within HIV services and strengthening referral pathways to reduce vision-related morbidity.

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PREVALENCE AND GENOTYPIC CHARACTERISTICS OF ANAL INFECTION WITH HIGH-RISK HUMAN PAPILLOMAVIRUS IN HIV-POSITIVE MEN WHO HAVE SEX WITH MEN

Alejandro Benjhamel Flores Argote[1], Tania Vargas Rivero[1], Gustavo Eduardo Allende Larraín[1], Amilcar Alejandro Flores Leon[2]

[1]Universidad Mayor de San Simón, Cochabamba, Plurinational State of Bolivia, [2]Univ Mayor of San Simon, Cochabamba, Plurinational State of Bolivia

Human papillomavirus (HPV) infection in the anal region is highly prevalent among men who have sex with men (MSM), especially those living with HIV (Human Immunodeficiency Virus) positive. This situation increases the risk of developing precancerous lesions and anal cancer, making this group a priority for prevention and early detection strategies. According to the ICO/IARC Information Centre (2023), there are no epidemiological data in Bolivia on the prevalence of anal HPV in men, highlighting an important information gap. In this context, the present study constitutes the first of its kind conducted in the country. The objective was to estimate the prevalence of high-risk HPV (HR-HPV) in the anal region and to identify the predominant genotypes among MSM attending the Igualdad Community Center in the city of Cochabamba, Bolivia. This was a quantitative, observational, descriptive, cross-sectional study involving 73 HIV-positive MSM participants. Biological samples were collected according to the sexual practices reported by the participants. Detection and genotyping of HR-HPV (14 genotypes) were performed using Anyplex RT-PCR. The results showed an anal HR-HPV prevalence of 80.7%, with the most frequent genotypes being HPV-31 (34.8%), followed by HPV-51 (30.4%), HPV-58 (30.4%), and HPV-39 (26.1%). A clear predominance of multiple infections (≥2 genotypes) in the anal region was also observed (82.6%). Prevalence was higher in younger age groups, with the greatest concentration among individuals between the ages of 23 and 27 years. In conclusion, the prevalence of anal HR-HPV infection in this population is very high. Therefore, it is necessary to strengthen comprehensive prevention strategies, including timely vaccination, sexual health education, and the promotion of protective methods. It is also essential to implement screening and early diagnosis programs targeted at this population.

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INCIDENCE AND RISK FACTORS FOR DEVELOPMENT OF METABOLIC SYNDROME AMONG NEWLY DIAGNOSED PEOPLE LIVING WITH HIV : A MULTICENTER FOLLOW UP STUDY, GREATER ACCRA REGION, GHANA, 2025

Magdalene Akos Odikro[1], Kwasi Torpey[1], Margaret Lartey[1], William Kwame Witts[1], Elijah Painstil[2], Ernest Kenu[3]

[1]University of Ghana, Accra, Ghana, [2]Boston University Chobanian & Avedisian School of Medicine, Boston MA, USA, MA, United States, [3]Univ of Ghana, Accra, Ghana

HIV related inflammaging and ART usage predisposes People Living with HIV (PLWH) to Metabolic Syndrome (MetS) - A clustering of metabolic disorders that increases the likelihood of developing diabetes, cardio-vasular and kidney diseases. Longitudinal data on MetS and it's risk factors are sparse in Ghana. We determined the incidence and risk factors for development of MetS among newly diagnosed PLWH in Accra. A 12-month prospective study enrolled and followed up newly diagnosed adults ≥ 18 years without MetS from four ART sites in the Greater Accra. Demographic and lifestyle data, blood pressure, waist circumference, fasting blood sugar, and lipids were measured at months 0, 6 and 12 using WHO's Steps Survey approach. MetS was defined using the Joint Consensus Criteria as the presence of any three sub-components; central obesity, raised blood pressure, impaired fasting glucose, reduced high-density lipoprotein cholesterol or raised triglycerides. MetS incidence rate was estimated and the Weibull Proportional-Hazards (PH) model for interval censored data was fit for multivariate risk factor identification. Of 294 participants without MetS followed up over the 12-month period, 79 developed MetS with incidence rate of 49 per 100 person years (95% CI: 37.9-53.1). Overall time at risk was 1,917 Person Years with Mean time to development of MetS being 5.5 months (SD: 2.83). Participants aged 50 years and above had 4.5 times the risk of developing incident MetS compared to those 25 years and below (aHR=4.5, 95% CI: 1.9-10.7), females had 3.8 times the risk of developing MetS compared to males (aHR= 3.8 ,95% CI: 2.1-6.7) and obese individuals had 2.6 times the risk of developing MetS compared to healthy individuals (aHR= 2.6, 95% CI: 1.1-6.3). We estimated a high incidence of MetS among newly diagnosed PLWH. Older age, female sex and obesity were risk factors for development of incident MetS. Metabolic screening should be intensified in the first year of HIV diagnosis with prioritisation of females, older and obese individuals.

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LEVERAGING LARGE LANGUAGE MODELS (LLMS) APPLIED TO MYCOBACTERIUM TUBERCULOSIS GENOMIC MUTATIONS FOR PREDICTING DRUG RESISTANCE: A PROOF OF CONCEPT STUDY USING CRYPTIC DATA

Mamadou D. Coulibaly, Hamady Coulibaly, Ibrahim Sanogo, Fousseyni Kane, Aminata Traore, Cheick Oumar Tangara, Seydou Doumbia

University Clinical Research Center, Mali, Bamako, Mali

Drug-resistant tuberculosis (DR-TB) remains a major public health challenge. Conventional genotypic prediction of drug resistance relies primarily on curated mutation catalogues or classical machine learning (ML), which often fail to capture the combinatorial and contextual complexity of genomic variation. LLM, which treat sequences as "biological text," offer a novel paradigm for learning nuanced mutation patterns and longrange dependencies, potentially enhancing resistance detection in *Mycobacterium tuberculosis*. Whole-genome sequencing (WGS) data from the CRyPTIC consortium were processed using a DuckDB-based streaming pipeline to extract key resistance associated mutations in rpoB, katG, and pncA. Mutations were encoded as textual tokens to create a semantic representation of each isolate. DistilBERT, a LLM architecture, was fine-tuned separately for rifampicin (RIF) and isoniazid (INH) resistance prediction using binary resistant/susceptible (R/S) phenotypes. A Random Forest model served as baseline. Model interpretability was examined through attention maps highlighting influential genomic positions. The LLM achieved

strong predictive performance (RIF eval_loss = 0.044 and INH eval_loss = 0.051), outperforming baseline accuracy (93% for RIF, 92% for INH) with superior calibration. Attention heatmaps consistently highlighted canonical resistance determinants (rpoB S450L, rpoB H445Y, and katG S315T) and revealed coattention structures illustrating contextual mutations interactions. In contrast, Random Forest feature importance emphasized simple mutation counts, lacking the depth captured by LLM. LLMs effectively learn genomic "language patterns" underlying TB drug resistance, offering superior contextual understanding, strong performance, and interpretable outputs. These capabilities position LLMs as scalable, explainable tools for next-generation genotypic diagnostic with potential to strengthen surveillance and guide personalized treatment, particularly in lowresource settings adopting WGS technologies.

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SPATIALLY-VARYING INTERRUPTED TIME SERIES METHODS FOR POPULATION-LEVEL PROGRAM EVALUATION

Niklas J.T. Hayden[1], Prakrut H. Kansara[2], Pamela R. Chacon-Uscamaita[3], Qianhui Zheng[3], Reinaldo O. Santos[4], Andres G. Lescano[5], Benjamin F. Zaitchik[2], Alisson F. Barbieri[4], William K. Pan[3], Mark M. Janko[1]

[1]University at Buffalo, Buffalo, NY, United States, [2]Johns Hopkins University, Baltimore, MD, United States, [3]Duke University, Durham, NC, United States, [4]Universidade Federal de Minas Gerais, Belo Horizonte, Brazil, [5]Universidad Peruana Cayetano Heredia, Lima, Peru

There is a need to evaluate public health interventions in low- and middle-income countries to ensure efficacy. Intervention data are frequently lacking from control programs. For example, routine vector-borne disease surveillance systems in the Amazon do not include data on intervention coverage and timing. Further, interventions for vector-borne diseases may have varying effects due to differences in implementation, coverage, or vector ecology. As a result, public health authorities lack information about whether interventions are working, and where. To address this problem, we develop spatially-varying interrupted time series methods and use malaria as a case study. The outcomes were *Plasmodium falciparum* and *Plasmodium vivax* cases. Cases were reported weekly across 321 municipalities in Brazil between January 2007 and December 2024 and 53 districts in Peru between January 2001 and December 2023. Intervention data was obtained from government and other published work for Peru and Brazil, respectively. Interventions were lagged by 1 year to account for a delay in implementation. We fit Bayesian interrupted time series models using Conditional Autoregressive prior distributions to allow intervention effects to vary spatially. Models were fit using Integrated Nested Laplace Approximation with a Poisson likelihood and were stratified by malaria species and adjusted for environmental factors. In Brazil, the effect of interventions on annual incidence (IRR) of *P. falciparum* ranged from 0.988 (95% CrI 0.986-0.990) to 1.003 (95% CrI 1.001-1.005) and *P. vivax* ranged from 0.943 (95% CrI 0.935-0.951) to 1.005 (95% CrI 0.998-1.012), depending on the area. In Peru, area-level intervention effects on *P. falciparum* ranged from 0.944 (95% CrI 0.916-0.969) to 1.003 (95% CrI 0.982-1.025) and *P. vivax* ranged from 0.990 (95% CrI 0.984-0.996) to 1.010 (95% CrI 1.004-1.016). The effectiveness of population-level malaria intervention programs varies spatially across the Amazon regions of Brazil and Peru. Next steps include evaluation of these programs considering land use and demographic factors.

7423

OUTCOMEFOCUSED IMPACT MODELING FOR CARDIOVASCULAR HEALTH SYSTEM STRENGTHENING IN VIETNAM: A REALWORLD APPROACH FOR LOW AND MIDDLEINCOME COUNTRIES (LMICS) DISEASEBURDEN REDUCTION

Romesh Wiss[1], Isabelle Lindenmayer[2], Ioana Ursu[3]

[1]Novartis Pharma AG, Basel, Switzerland, [2]Novartis Pharma AG, Novartis Pharmaceuticals Corporation, NY, United States, [3]Novartis Pharmaceuticals Ltd, London, United Kingdom

Low- and middle-income countries (LMICs) face a dual burden of persistent infectious diseases and growing non-communicable diseases (NCDs), such as cardiovascular disease (CVD). As NCDs drive morbidity and deaths, there is a need to identify which health system strengthening (HSS) interventions improve equitable long-term health outcomes. Most HSS programs are evaluated with activity-based metrics, limiting understanding of population-level and economic impact and hindering governmental intervention planning. Cùng Sống Khỏe (CSK) is a large-scale HSS program implemented in rural Vietnam to bolster primary care delivery for CVD prevention and treatment. Interventions included community-based screening, improved referral pathways, patient and provider education, and structured follow-up. CSK screened 117,787 more individuals and linked 8,877 more patients to treatment. From pre to postintervention, hypertension control improved from 32% to 41% and glycemic control from 27% to 39%. To move past activity-based evaluation, we developed an outcome-focused impact assessment to translate observed improvements in disease control into projected long-term health and economic benefits. A health-economic model simulated transitions across CVD health states using CSK's pre- and post-intervention data. Outcomes included deaths, disability-adjusted life years (DALYs), non-fatal CVD events, and cost. Over 10 years, the model projected 315 CVDrelated deaths averted (13.9% reduction), 3,977 DALYs averted (6.6% reduction), 588 CVD events avoided (7.4% reduction), and $1,477 incremental cost-effectiveness ratio (ICER) per QALY gained (versus $14,637/QALY threshold). These findings highlight how integrated, equity-focused HSS interventions can reduce LMIC NCD burden and show a scalable approach to linking real-world implementation with population-level impact. Similar approaches are being tested in South Africa, Rwanda, and Mexico.

7424

QUANTIFYING THE INVESTMENT GAP, COST OF MALARIA ELIMINATION, AND RETURNS ON INVESTMENT IN INDIA: A MACROECONOMIC ANALYSIS

Harsh Rajvanshi[1], Mrigendra Pal Singh[2], Praveen Bharti[3], Altaf Lal[4]

[1]Foundation for Disease Elimination and Control of India, Mumbai, India, [2]National Institute of Malaria Research, Jabalpur, India, [3]Jabalpur, India, [4]Sun Pharmaceuticals Industries Limited, India, Atlanta, GA, United States

Malaria continues to impose a significant health and economic burden in India, accounting for over half of cases and deaths in the WHO South-East Asia Region despite substantial progress in recent decades. Although India has committed to achieving zero indigenous malaria cases by 2027 and complete elimination by 2030 under the National Strategic Plan (NSP) 2023-2027, current investments may be inadequate to meet these targets. This study quantifies the economic burden of malaria, estimates the investment required for elimination, and assesses the potential returns on investment using a macroeconomic modelling approach. A district-level SEIR model stratified by endemicity (high, low, and malaria-free districts) was applied to estimate intervention costs under three scenarios: (1) present investment; (2) NSP 2023-2027-aligned investment; and (3) intensified interventions modelled on the Mandla Malaria Elimination Demonstration Project (MEDP) plus Sri Lanka and Bhutan-based approaches. The cost-of-illness method was used to estimate the national economic burden, including treatment costs, productivity losses, premature mortality, and personal protection expenditures. Forecasting models were applied to project malaria incidence

through 2035, while Disability-Adjusted Life Years (DALYs) and return on investment (ROI) were calculated to assess health and economic outcomes. The findings indicate that the current investment of approximately INR 6 per capita annually is insufficient. The required annual investment is estimated at INR 16,414 million, compared with INR 4,451 million allocated in 2024. Under current trends, malaria transmission would persist beyond 2035. Scaling up to NSP targets could reduce cases to two digits by 2032, eliminate deaths by 2029, and yield an ROI of 3.18. The intensified MEDP-plus scenario projected near elimination by 2030, zero deaths by 2028, and a higher ROI of 3.76. In conclusion, these findings demonstrate that closing India's malaria investment gap is both a public health and macroeconomic priority.

7425

AI-EMPOWERED PLATFORM FOR MOBILE-ACQUIRED MICROSCOPY IMAGE ANALYSIS IN LOW RESOURCES SETTINGS

Emna Harigua Souiai[1], Rafeh Oualha[2], Donia Driss[1], Nesrine Fekih-Romdhane[1], Yosser Zina Abdelkrim[3], Ikram Guizani[4]

[1]INSTITUT PASTEUR DE TUNIS, Tunis, Tunisia, [2]TUNIS, 11, Tunisia, [3]INSTITUT PASTEUR DE TUNIS, Ariana, Tunisia, [4]Inst Pasteur de Tunis, Tunis, Tunisia

Infectious diseases constitute significant global health challenges, requiring accurate diagnosis and therapeutic solutions. We aim at developing AI-based tool for the detection and quantification of intracellular parasites (i.e. Leishmania amastigotes) based on smartphone-acquired light microscopy images. The software will be deployed as a mobile application for use in low resources settings. We collected Giemsa-stained microscopy images of Leishmania-infected macrophages. We annotated pixels into three classes: host-cells, nucleus and amastigotes, exported in the COCO format. We implemented two models: (i) a modified architecture of U-Net with a pretrained VGG16 backbone was trained in two-phases: first as a binary classifier, then as a multi-class segmentation classifier, using the Dice loss as the loss function for both models, for its effectiveness in handling class imbalances and improving segmentation performance; and (ii) a fused framework for object detection based on YOLOv8 and the RF-DETR transformer model. We fine-tuned and optimized both model parts and then the fused framework, called ParaFusionNet.We acquired and annotated 180 Giemsa-stained microscopic images, containing 8,140 Leishmania amastigotes and 1,511 macrophages. Amastigotes accounted for only 0.65% pixels, highlighting the challenge of their detection and counting. First, we generated instance segmentation masks tailored for multiclass segmentation and bounding boxes for the object detection models. We split the dataset into training, validation and test sets (80%,10%,10%), and performed data augmentation. Overall, the VGG16-Unet model achieved satisfactory performances of 92% precision and 86% recall, while fine-tuned ParaFusionNet achieved 89% and 71%, respectively. We initiated model optimization strategies (e.g., pruning, quantization) to produce lightweight architectures tailored for deployment in a mobile application.Our Software shall serve a scalable user-friendly tool, enabling fast and accurate analysis of microscopy images in low resource settings.

7426

RAPID GLOBAL GROWTH AND INTENSIFICATION OF DENGUE SINCE 1990

Ahyoung Lim[1], Kishen Joshi[1], Preethi Lakshminarayanan[2], Katie Susong[1], Joseph Clarke[3], Jenicca Poongavanan[4], Talia M. Quandelacy[5], Ewerton Cousin[6], Houriiyah Tegally[4], Jue Tao Lim[7], Jonathan Mosser[8], Michael Johansson[9], Simon Iain Hay[10], Oliver Brady[2]

[1]London School of Hygiene & Tropical Medicine, LONDON, United Kingdom, [2]London, United Kingdom, [3]University of Cambridge, Cambridge, United Kingdom, [4]Stellenbosch University, Stellenbosch, South Africa, [5]CU Denver, Colorado School of Public Health, Aurora, CO, United States, [6]IHME/UW, Seattle, WA, United States, [7]Nanyang Technological University, Singapore, Singapore, [8]Institute for Health Metrics and Evaluation, University of Washington, Seattle, WA, United States, [9]Northeastern University, Boston, MA, United States, [10]University of Washington, Seattle, WA, United States

Dengue is an emerging infectious disease of growing global importance, yet the pace of its expansion remains poorly quantified because surveillance data are inconsistently reported across countries and over time. Addressing this gap requires both comprehensive data assembly and modelling approaches that can handle widespread missingness. Here we developed a hierarchical Bayesian spatio-temporal framework using integrated nested Laplace approximation (INLA) to impute and downscale dengue case counts for 143 countries between 1990 and 2024. Seasonality was captured through a global cyclic random walk backbone with smooth deviations by latitudinal band and country, enabling data-sparse countries to borrow strength from regional and global patterns while preserving local dynamics. We compared this approach against generalised additive models and Dirichlet-multinomial hierarchical models using cross-validation with artificially introduced data gaps of varying duration (2 weeks to 12 months). An automated data selection algorithm resolved discrepancies across multiple surveillance sources by prioritising the highest-quality record for each time-location combination. The INLA framework consistently outperformed alternatives across diverse gap scenarios. Applying this framework, we estimate 73.9 million reported dengue cases worldwide over this period, including 10.6 million cases not captured in existing WHO databases. Reported incidence increased 39-fold, with growth fastest in recently emerging regions in Africa, the Middle East and South Asia. Outbreaks have doubled in cyclical strength and become increasingly synchronised globally. This modelling framework provides a generalisable approach for reconstructing incomplete surveillance time series and offers a foundation for improved prediction and control of dengue during a period of declining incidence for most other infectious diseases.

7427

THE GLOBAL DENGUE OBSERVATORY: A PUBLIC PLATFORM FOR REAL-TIME MULTI-NATIONAL DENGUE SURVEILLANCE AND SEASONAL CONTEXT

Katie Milligan Susong[1], Ahyoung Lim[2], Har-Kishen Joshi[3], Oliver Brady[3]

[1]London School of Hygiene and Tropical Medicine, London, United Kingdom, [2]London School of Hygiene & Tropical Medicine, LONDON, United Kingdom, [3]London, United Kingdom

Dengue virus is the largest growing mosquito-borne threat globally, yet timely awareness remains hampered by fragmented surveillance, heterogeneous reporting delays, and prolonged data gaps across countries. Existing international dashboards provide a valuable but incomplete picture, as reporting lags of weeks to months are common, and seasonal context is rarely provided. We developed the Global Dengue Observatory (GDO) (globaldengueobservatory.org), an open, public-facing platform that integrates dengue case data from more than 70 countries across eight world regions into a single harmonised resource. The GDO combines data from three international surveillance sources: the WHO Global Dengue Dashboard, the WHO South-East Asia Region Dashboard, and the Pan American Health Organisation dataset with historical records from the OpenDengue database. A standardised process harmonises these sources, applies empirical reporting-delay corrections where data permit, and uses a season-aware proportion-based nowcasting method to fill gaps in recent months. The resulting outputs allow users to see, at a glance, how the current dengue season compares to historical norms; through monthly and cumulative case estimates, ratios relative to a country's typical seasonal trajectory, and severity classifications that highlight countries experiencing unusual activity. These indicators are presented through interactive global, regional, and country-level visualisations designed for broad accessibility to national decision-makers, researchers, and the general public. The GDO is updated regularly, and all processed data and source code are publicly available, enabling independent analysis and local adaptation. By consolidating fragmented reporting into a single open resource with consistent seasonal context, the GDO addresses a critical gap in global

dengue situational awareness, supporting timely risk communication, outbreak response prioritisation, and evidence-based advocacy for dengue control investment.

7428

ASSESSING THE RELATIONSHIP BETWEEN CONTACT AND TRAVEL PATTERNS USING A MULTI-YEAR HOUSEHOLD COHORT IN GUANGZHOU, CHINA

Kathryn M. Hogan[1], Jonathan M. Read[2], Claire P. Smith[3], Justin Lessler[3], Amy Wesolowski[4], Shaun Truelove[1], Joanne Katz[1], Kin On Kwok[5], Chao Qiang Jiang[6], Steven Riley[7], Derek Cummings[8]

[1]Department of International Health, School of Public Health, Johns Hopkins University, Baltimore, MD, United States, [2]Centre for Health Informatics, Computing, and Statistics, Lancaster Medical School, Lancaster University, Lancaster, United Kingdom, [3]Department of Epidemiology, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States, [4]Department of Epidemiology, School of Public Health, Johns Hopkins University, Baltimore, MD, United States, [5]JC School of Public Health and Primary Care and Stanley Ho Centre for Emerging Infectious Diseases, The Chinese University of Hong Kong, Hong Kong, Hong Kong, [6]Guangzhou Twelfth People's Hospital, Guangzhou, China, [7]MRC Centre for Global Infectious Disease Analysis, Imperial College London, London, United Kingdom, [8]Department of Epidemiology, School of Public Health, and Department of Biomedical Engineering, School of Engineering, Johns Hopkins University, Baltimore, MD, United States

Human mobility and social contact patterns jointly shape respiratory pathogen spread but are often used independently in infectious disease transmission models. The empirical relationship between an individual's contact patterns and mobility behaviors is poorly characterized. We used contact diaries with geocoded locations for each contact-event from a cohort in Guangzhou, China to quantify how daily travel relates to contact patterns. For each person-day, we approximated the minimal distance in kilometers (km) between all locations where reported contacts were made and individual's home location and collected data on the total number of contacts a person reported in that day. The outcome of interest was total number of contacts per person-day. We used mixed-effects negative binomial regression, with random intercepts for individual and household nested within village, adjusted for age group, sex, visit, weekday/weekend, and a categorical variable for the number of contact-encounters reported that day. There were 29,622 contact-events with location data included in the analytic sample of 2,988 participants from 1,158 households recorded over three visits between 2011 and 2019. Median contacts per person-day were 10 (Interquartile Range: 6-19). Participants were categorized into five distance groups ranging from no travel (0 km) to high mobility (9.25-2,937 km), with median distances of 0, 0.21, 1.32, 4.58, and 20.8 km respectively. Compared with the reference group who did not travel any distance, distance traveled was associated with progressively higher contact rates. For total contacts the adjusted contact rate ratios (aCRR) were: very low distance aCRR = 2.36 (95% CI 2.04-2.74), low = 2.72 (95% CI 2.36-3.14), medium = 3.03 (95% CI 2.63-3.50), and high = 3.06 (95% CI 2.65-3.54). Greater daily travel was associated with higher numbers of total contacts. These results show that incorporating observed mobility-contact relationships into epidemiological models may improve representation of behavioral heterogeneity in transmission.

7429

TRAVELLING WAVES AND SYNCHRONY OF DENGUE DYNAMICS AT THE FINEST SPATIAL SCALE OF A HYPERENDEMIC REGION IN VIETNAM

Thao Nguyen[1], Wala Areed[1], Dan Weinberger[2], Eloise Skinner[1], Kien Quoc Do[1], Dung Phung[1]

[1]The University of Queensland, Herston, Australia, [2]Yale University, New Haven, CT, United States

Dengue remains a major global health threat, causing high mortality and morbidity annually, with transmission strongly affected by climate change, urbanization and human mobility. However, the spatiotemporal dynamics of dengue at the fine spatial scales are rarely known. This study examines the seasonal and multiannual dengue trend and travelling waves across the Mekong Delta Region (MDR), a hyperendemic area in Vietnam. Monthly dengue cases data were collected from 555 communes (the finest spatial scale) in the MDR from the national surveillance system, ranging from 2010 to 2025. Population data were collected from the General Statistics Office in Vietnam and estimated with the annual birth rate. We used wavelet analysis to isolate the seasonal (10-14 months), biennial (15-30 months), and multiannual (31+ months) patterns. For each location, we assessed phase angles and differences in timing of dengue cycles between 153,735 location pairs to identify the travelling waves. Dengue epidemic peaks occurred mostly in the early months of the rainy season, with 99% of communes exhibiting clear seasonality. Strong synchrony in dengue dynamics was observed across the MDR at both annual and multiannual scales. Epidemic amplitudes were highest in coastal and urban areas. Approximately 14% of communes experienced earlier epidemic peaks, forming small clusters of 2-3 neighbouring communes dispersed throughout the region. The earliest epidemic was recorded at a coastal commune. These findings reveal the spatiotemporal dynamics of dengue at the finest scales to inform practitioners targeting interventions and optimizing resource allocation. This study also supports early warning system development. Future work should focus on identifying determinants affecting dengue transmission.

7430

VALIDATION STUDY COMPARING FIVE PGP3-BASED PROTOTYPES TO SUPPORT IN-COUNTRY LABORATORY TESTING OF DRIED BLOOD SPOTS FROM TRACHOMA SURVEYS

Madison L. Bearden[1], Ambahun Chernet[2], Getahun Ayenew[2], Jean Saunders[3], Quynh Do[3], Matthias Schwarz[3], Sarah Gwyn[4], E. Kelly Callahan[1], Tomoaki Uchiki[5], Diana L. Martin[4], Scott D. Nash[1], Marco A. Biamonte[3]

[1]The Carter Center, Atlanta, GA, United States, [2]The Carter Center, Bahir Dar, Ethiopia, [3]Drugs & Diagnostics for Tropical Diseases (DDTD), San Diego, CA, United States, [4]Centers for Disease Control and Prevention, Atlanta, GA, United States, [5]Medical & Biological Laboratories, Co. Ltd., Tokyo, Japan

Serological monitoring is becoming common within trachoma control programs. Much of the early evaluation of trachoma serology within endemic countries was conducted using a lateral flow assay (LFA) developed by the United States Centers for Disease Control and Prevention (CDC). The Pgp3 wet-conjugate dipstick LFA had virtually identical test performance as the Pgp3 multiplex bead assay (MBA): 92% sensitivity; 98% specificity, and limit of detection similar to the MBA (75 vs 57 ng/ml). In collaboration with CDC and The Carter Center, Drugs & Diagnostics for Tropical Diseases (DDTD) has developed several Pgp3-targetted prototypes based on CDC's LFA, to create a manufacturable and commercial dipstick test available to programs. The aim of this study was to evaluate assay performance of five DDTD LFA prototypes compared to the CDC LFA within an endemic-country laboratory. In December 2025, a validation subset (N=70) of DBS was constructed from those collected from a survey in Tach Gaynt district, Amhara, Ethiopia, in 2023. The subset was selected to preserve the underlying age distribution of all samples. Five tests were evaluated at the Amhara Public Health Institute: (A) the CDC wetconjugate dipstick, (B) the DDTD Construct 1 (C1) dryconjugate dipstick, (C) the DDTD Construct 2 (C2) dryconjugate dipstick, and (D/E) the DDTD C1/C2 dryconjugate test strips placed in cassettes without a blood filter. Three DBS snips from the same participant (40 positives/10 negatives) were used to evaluate the three dipsticks, and two DBS snips from the same participant (15 positives/5 negatives) were used to evaluate the two cassettes. Concordance with historical positive/negative data from the CDC's LFA was high, with slight variation observed. The C1 dipstick performed best with a 93% (37/40) positive and 90% (9/10) negative concordance while the C2 dipstick had 88% (35/40) positive and 90% (9/10) negative concordance. The C1 cassette performed better than the C2 cassette with 100% positive/negative concordance. Within this setting, the C1 dipstick had the best performance and will continue to be developed and integrated into trachoma programs.

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OPTIMIZATION OF A SEROLOGICAL RAPID TEST TO DETECT ANTIBODIES TO PGP3 IN LABORATORY AND POINT-OF-CARE SETTINGS FOR TRACHOMA SURVEILLANCE

Quynh Do[1], Jean Saunders[1], Rhea Perez[2], Matthias Schwarz[1], Tomoaki Uchiki[3], Tomoaki Taniguchi[3], Junichi Akatsuka[3], Scott D. Nash[4], Madison L. Bearden[4], Marco Biamonte[1]

[1]Drugs & Diagnostics for Tropical Diseases (DDTD), San Diego, CA, United States, [2]Adelante Bio, San Diego, CA, United States, [3]Medical and Biological Laboratories Co., Ltd. (MBL), Tokyo, Japan, [4]The Carter Center, Atlanta, GA, United States

Trachoma, caused by the bacterium *Chlamydia trachomatis,* is the leading infectious cause of blindness worldwide. Serological surveillance in children aged 1 to 5 years is critical for monitoring transmission and guiding programmatic decisions. A lateral flow assay detecting antibodies to the *C. trachomatis* antigen Pgp3 was developed at the United States Centers for Disease Control and Prevention (CDC) and demonstrated comparable performance to the reference multiplex bead assay (MBA). Using the CDC's Pgp3 antibody test as the foundation, we developed a simplified trachoma dipstick with integrated dried conjugate, eliminating cold-chain requirements and reducing time to result. Development focused on antigen expression and purification, scaleup of visual detector nanoparticles, and cover tape incorporation, resulting in an optimized dipstick that can be used in low-resource settings in under 60 minutes. Prototypes were evaluated at the CDC using 100 banked and de-identified dried blood spot (DBS) samples, demonstrating 100% concordance with MBA data (51/51 negative, 49/49 positive). Accelerated stability studies showed consistent performance for at least 8 weeks at 55°C, projecting shelf life of ≥3 years at 25ºC or ≥8 months at 40ºC. A cassette version of the antibody test was also developed for fingerprick blood at the point of care. Matrix equivalence studies using contrived plasma, whole blood, and DBS samples demonstrated comparable performance between cassette and dipstick versions across all sample types. Manufacturing has been scaled up for high-throughput production of 100,000 tests per lot with an estimated $2 ± 0.5 USD per test. The first production lot of 30,000 tests demonstrated strong concordance with MBA data (48/49 negative, 49/51 positive; kappa = 0.94) as determined at the CDC. These tests are being used to support trachoma surveillance programs in multiple African and South American countries, in partnership with Sightsavers and The Carter Center. Next steps include pilot lot manufacturing under ISO 13485 standards to support validation and verification studies, with the goal of programmatic adoption in 2028.

7432

PREDICTING TRACHOMATOUS INFLAMMATION: FOLLICULAR RISK IN ETHIOPIAN CHILDREN AGED 1—9 YEARS USING INDIVIDUAL LEVEL DATA FROM TROPICAL DATA SURVEYS: A RANDOM FOREST AND PENALIZED LOGISTIC REGRESSION APPROACH

Dawit Getachew Assefa[1], Rhys Peploe[2], Genet Kiflu[3], Tesfahun Bishaw[3], Tsegaye Alemu[3], Fikreab Kebede[3], Anthony W Solomon[4], Emma Harding-Esch[5], Philippe J Guerin[2], **Prabin Dahal**[6]

[1]Dilla University, Dilla, Ethiopia, [2]University of Oxford, Oxford, United Kingdom, [3]Department Disease Prevention and Control Lead, Ministry of Health, Addis Ababa, Ethiopia, [4]Department of Control of Neglected Tropical Diseases, World Health Organization, Switzerland, Switzerland, [5]London School of Hygiene & Tropical Medicine, London, United Kingdom, [6]IDDO, University of Oxford, Oxford, United Kingdom

Trachoma remains a leading infectious cause of blindness, particularly among children in low-resource settings. In Ethiopia, limited access to water, sanitation, and hygiene (WASH) sustains transmission. Although extensive trachoma survey data exist, translating these into tools to identify high-risk communities and guide interventions remains challenging. This study estimated trachomatous inflammation—follicular (TF) prevalence at the evaluation unit (EU) level among children aged 1-9 years and developed predictive models for TF risk using demographic and WASH factors (2010-2025). Harmonized TF1-9 survey data from Ethiopia (2010-2025) were analyzed. WASH predictors included time to drinking and washing water, soap availability, latrine access, and a composite deprivation index. The outcome was individual-level TF status. Penalized logistic regression and random forest models predicted TF probability using demographic and WASH variables. Models used group-based 5-fold cross-validation at the EU level to account for clustering. Performance was assessed using calibration. A total of 608,367 children from 999 clusters across 1,234 EUs were included; median age was 5 years (IQR 3-7), and 50.9% were male. Drinking water collection time was <30 min in 28.9%, 30-59 min in 25.1%, and ≥60 min in 46.0%; for washing water: 32.9%, 23.2%, and 43.9%. Soap was available in 2,878 (8.2%) households. TF prevalence was 8.5%. Most EUs had TF <5% (659), 211 had 5-9.9%, and 351 had ≥10%. In EU-based cross-validation, the random forest model shows strong predictive performance (Brier score = 0.057). Variable importance identified age as the strongest predictor of TF, followed by water access and soap availability; sex and wash deprivation contributed minimally. In conclusion, Predictive modeling can support programmatic decision-making by identifying high-risk EUs without requiring exhaustive surveys. Findings highlight the importance of improving water access and hygiene in sustaining trachoma control and suggest integrating data-driven targeted approaches may enhance efficiency toward elimination.

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ABSENCE OF KNOWN MACROLIDE RESISTANCE-ASSOCIATED MUTATIONS IN OCULAR *CHLAMYDIA TRACHOMATIS* FOLLOWING 15 YEARS OF MASS DRUG ADMINISTRATION IN AMHARA, ETHIOPIA

Scott Nash[1], Magdalena Capó Bauza[2], Inge van Loo[2], Mayk Luchessi[2], Ambahun Chernet[3], Mohammed Feyisso[3], Adisu Abebe[4], Tania Gonzalez[1], Eshetu Sata[3], E Kelly Callahan[1], SA Morré[5], Christian Hoebe[2], Petra Wolffs[2]

[1]The Carter Center, Atlanta, GA, United States, [2]Maastricht University Medical Centre+, Amsterdam, Netherlands, [3]The Carter Center, Addis Ababa, Ethiopia, [4]Amhara Regional Health Bureau, Bahir Dar, Ethiopia, [5]Maastricht Care and Public Health Research Institute (CAPHRI), Maastricht University, Amsterdam, Netherlands

In areas experiencing persistent trachoma, countries have begun to introduce more frequent than annual (MFTA) mass drug administration (MDA). Where repeated rounds of MDA are administered, a concern is whether azithromycin exposure could select for antimicrobial resistance in *Chlamydia trachomatis* (*Ct*), potentially contributing to the ongoing transmission in persistent districts. In 2023, the trachoma control program in Amhara, Ethiopia tested a MFTA strategy calling for a community-wide MDA followed by a child-only MDA 4 weeks later in persistent districts Lasta and Wadilla. This study's aim was to monitor the emergence of azithromycin resistance through the 23S rRNA gene assay within a population-based sample in Lasta and Wadilla prior to and immediately after MFTA MDA. Surveys were conducted in Lasta and Wadilla in September 2022 after 13 annual rounds of MDA, and again in July 2023, 3 weeks after the MFTA (round 14 and 15) MDA. Children ages 1-5 years were swabbed in 2022, and children ages 1-9 years were swabbed in 2023. All positive samples from Lasta (2022/2023) and Wadilla (2023) were shipped to the Maastrict University Medical Center+. Among the 2,045 samples collected, 58 were positive in Lasta in 2022, and 29 were positive in Lasta and 14 were positive in Wadilla in 2023. 91/100 swabs were able to be sequenced. Analysis of the 23S rRNA gene revealed that no mutations occurred in the specified resistance-associated mutations (RAMs) positions: A2057, A2058, A2059, C2452 and T2611 (*E. coli* numbering) in *Ct* samples collected prior to or directly after MFTA MDA. No additional mutations were detected, the 23S sequences were nearly identical in samples across the two districts and timepoints. Sequences of the genes encoding the L4 and L22 ribosomal proteins were highly conserved, with only a small number of point mutations observed and no clear association with azithromycin resistance. Despite 13-15 MDA rounds, we detected no known RAMs in the *Ct* strains analyzed,

even among samples collected 3 weeks after MFTA MDA. Azithromycin resistance is unlikely to be a major contributor to ongoing trachoma transmission in this setting.

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CHARACTERIZING SEPSIS AMONG I ASSOCIATED UNDER-FIVE MORTALITY IN WESTERN KENYA

Lavender Apondi Owiti[1], Harun Odhiambo Owuor[2], Fredrick Onduru[1], Edwin Kiplagat[1], Broline Sagini[1], Sarah Hawi Ngere[3], Dickson Gethi[1], Aggrey Kitiezo Igunza[4], Hellen Muttai[1], Victor Akelo[5]

[1]Kenya Medical Research Institute (KEMRI), Kisumu, Kenya, [2]kemri, Kisumu, Kenya, [34]KEMRI - CGHR, Kisumu, Kenya, [5]U.S. Centers for Disease Control and Prevention, Kisumu, Kisumu, Kenya

Klebsiella pathogen is a key contributor to bacterial infection and under-five deaths globally, yet its role in sepsis within the causal chain of child deaths remains poorly defined. We quantified the burden and characteristics of sepsis among *Klebsiella*-related under-five mortality in Western Kenya. We analysed Child Health and Mortality Prevention Surveillance (CHAMPS) data between 2017 and 2025, with laboratory-confirmed *Klebsiella* pathogen from culture and Taqman array card testing. Causes of death (COD) were determined by a panel of experts using data from post-mortem examinations conducted through minimally invasive tissue specimen testing, clinical records, and verbal autopsy. We compared sepsis and non-sepsis deaths using chi-square and Wilcoxon rank-sum test. Among 682 *Klebsiella*-associated deaths, 265 (38.9%) had sepsis in the causal chain. Sepsis burden was significantly higher among early (58.6%) and late neonates (71.4%). Sepsis was more frequent in rural settings (44.7%) than Peri-urban settings (31.8%) (p>0.001). Both prolonged (≥72 hours) and absence hospitalization were associated with a higher proportion of sepsis deaths (~43%) compared to short hospital stays (<72 hours: 11.5%) (p<0.001). Malnutrition in the causal chain was more common among sepsis deaths (57.7% vs 42.3%, p<0.001). There were no significant differences observed by sex, HIV status, birth weight, sickle cell status, or mode of delivery. Sepsis is present in nearly two in five *Klebsiella*-associated child deaths and disproportionately affects neonates. The observed pattern by residence, malnutrition, and prolonged hospitalization underscores the need for early identification and targeted interventions in the community and facility settings. Targeted strategies to improve early recognition, infection prevention, and management of high-risk neonates and malnourished children are essential to reduce sepsis-related mortality in similar settings.

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BLOOD CULTURE POSITIVITY, PATHOGENS, AND CLINICAL OUTCOMES AMONG YOUNG INFANTS WITH POSSIBLE SERIOUS BACTERIAL INFECTION IN PAKISTAN

Fatimah Azhar

The Aga Khan University, Karachi, Pakistan

WHO guidelines for management of possible serious bacterial infection (PSBI) in young infants in low-resource settings relies on syndromic diagnosis, with limited microbiological confirmation. We conducted secondary analysis of blood culture and clinical outcomes from hospitals in Pakistan participating in two randomized controlled trials evaluating simplified antibiotic regimens in infants aged 0-59 days. Among 2440 infants (1125 with single low-mortality-risk sign in RCT1; 799 with moderate-mortality-risk in RCT2; and 516 screened but not enrolled in RCT2 after 48-hours of ampicillin and gentamicin), blood cultures were obtained from 2364 (96.8%). Culture positivity was 7.4% in low-mortality-risk infants and 9.4% in moderate-mortality-risk infants, compared with 20.9% among non-enrolled infants. After excluding contaminants, pathogen-confirmed infections occurred in 2.4%, 3.2% and 12.7% respectively. Non-enrolled infants showed higher culture positivity despite prior antibiotics, suggesting a higher-risk group enriched for true bacteremia. Gram-negative organisms predominated (57.6%-78.0%) with frequent multidrug resistance and reduced susceptibility to commonly used antibiotics. Clinical signs did not predict bacteremia among low-mortality-risk infants. Among moderate-mortality-risk infants, culture-positive infants were younger (mean age 16.3±18.4 vs 24.2±19.2 days), had lower weight-for-age z-scores (–1.77±1.00 vs –1.48±1.00), and were more frequent in non-enrolled infants (71.6%) than enrolled infants (28.4%). Poor clinical outcomes by day 15 were infrequent but higher among culture-positive infants (11.5% compared with 6.8% in low-mortality-risk infants and 3.4% compared with 2.2% in moderate-mortality-risk infants). Bloodstream infections in young infants with PSBI are uncommon but clinically important. Despite low culture positivity and generally favorable outcomes, these findings highlight limitations of syndromic diagnosis and support integrating microbiological surveillance with risk stratification to guide empiric therapy and strengthen antimicrobial stewardship.

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SEROSURVEILLANCE AND ACTIVE CASE DETECTION IN A LEPROSY COHORT: LESSONS LEARNED AND PUBLIC HEALTH IMPACT

Nithya Narayanaswamy[1], Audra Bass[1], Erica Magueta Silva[2], Maisa Pereira Vieira[2], Daisy C. Monteiro[3], Lorena P Oliveira[4], Pedro H. Marcal[5], Alexandre Branco[6], Shaiana Fraga Oliveira[1], Julie Clennon[7], Lance Waller[7], Jeffrey M. Collins[8], Thomas R. Zeigler[9], Marcos Daniel Silva Pinheiro[4], Lucia Fraga[10], Jessica K. Fairley[7]

[1]Rollins School of Public Health, Atlanta, GA, United States, [2]Programa of Multicêntrico de Bioquímica e Biologia Molecular/PMBqBM, Universidade Federal de Juiz De Fora-Campus Governador Valadares, Governador Valadares, Brazil, [3]Programa of Multicêntrico de Bioquímica e Biologia Molecular/PMBqBM, Universidade Federal de Juiz De Fora-Campus Governador Valadares, Governador Valedares, Brazil, [4]Univale, Governador Valedares, Brazil, [5]Georgia Institute of Technology, Atlanta, GA, United States, [6]Centro de Referência em Doenças Endêmicas e Programas Especiais, Governador Valedares, Brazil, [7]Emory University, Atlanta, GA, United States, [8]Emory University School of Medicine, Department of Infectious Disease, Atlanta, GA, United States, [9]Emory University School of Medicine, Department of Endocrinology, Atlanta, GA, United States, [10]Uniformed Services Univ of the Health Sciences/ UNIV ALE/DRS, Bethesda, MD, United States

Brazil bears a disproportionate burden for Hansen's disease (leprosy), reporting over 10,000 new cases in 2023. Conventional surveillance methods rely predominantly on passive clinical case detection, often missing subclinical infections. Anti-LID-1 and PGL-1 seropositivity and environmental surveillance offer surrogate markers of early subclinical infection and exposure, but evidence on implementing serosurveillance models remains limited. We conducted a mixed cross-sectional and prospective longitudinal study incorporating serosurveillance and active case detection across four endemic municipalities in Minas Gerais, Brazil. A total of 1,315 individuals underwent multiplex bead immunoassay (MBA) serological testing for anti-LID-1 antibodies alongside helminth co-infection markers, questionnaire, and clinical evaluation. LID-1 positive participants were enrolled into a nested matched longitudinal cohort with seronegative controls. Implementation outcomes were assessed retrospectively using the Proctor et al. framework. A prior history of Hansen's disease was reported by 72 participants (5.5%), while 94 (7.2%) tested seropositive for anti-LID-1 antibodies. Seropositivity varied by municipality (Mantena 12%, Inhapim ~4%) and was more common among rural residents. All 77 eligible seropositive participants were enrolled into the longitudinal cohort, with 1:1 matching achieved for 76 (98.7%). Active follow-up identified 19 incident HD cases (18 LID-1 positive, 1 LID-1 negative), predominantly borderline leprosy (53%, 10/19), with case detection highest at first follow-up (8/19). Pragmatic adaptations included a multi-step clinical screening approach documented within the Proctor et al. fidelity framework. Multicomponent serosurveillance incorporating MBA serology, spatial targeting, environmental surveillance, and longitudinal follow-up is feasible in endemic Brazilian municipalities. These findings support early intervention by identifying subclinical infection before clinical disease develops, and provide a replicable model for leprosy serosurveillance in endemic settings.

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PROOF OF CONCEPT OF A SENSITIVE, RAPID, AND ECONOMICAL ONE-POT CRISPR DETECTION TEST FOR *COXIELLA BURNETII*

Cecilia Y. Kato[1], Misbah Rahman[2], Gilbert Kersh[3]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Johns Hopkins University Department of Molecular Microbiology and Immunology, Baltimore, MD, United States, [3]Centers for Disease Control, Atlanta, GA, United States

Coxiella burnetii (*Cb*), a potential biothreat agent and highly infectious select agent, causes Q-fever. Q-fever has a worldwide distribution and the primary reservoirs are livestock. Outbreaks of Q-fever may therefore occur in farms and *Cb* is mainly transmitted to humans through inhalation of contaminated aerosols. *Cb* molecular testing is typically performed in centralized research or reference laboratories. To potentially conduct effective environmental and livestock Q-fever testing at the location of the investigation, we developed a rapid and sensitive method that is easily portable and cost effective. The method does not require traditional DNA extraction and consists of a simplified sample lysis/inactivation step followed by a single tube preparation of low volume reactions for a 15-minute isothermal amplification and a 15-minute CRISPR/Cas12a detection reaction, that are separated by a vapor barrier and combined by a quick spin between incubations. Visualization is performed by ultraviolet or blue light illumination in a shaded box and camera (smartphone) capture of results. The assay targets the IS1111 transposase insertion sequence present in *Cb* strains at various copy numbers. Using the Nine Mile phase II (NM ph2) strain at 20 IS1111 repeats/genome equivalent (ge), the lower limit of detection is 2 ge per reaction. Specificity testing included three sequence types found in the United States (ST-8, ST-16, and ST-20) and excluded *Legionella pneumophila* and *Francisella tularensis* near neighbors. NM ph2 spiked environmental swab eluate and goat vaginal swab eluate were consistently positive as low as 10 ge per reaction. In conclusion, the laboratory performance of the *Cb* CRISPR detection system shows promise for field usage as it is sensitive, specific, economical and easy to perform, providing timely results at the point of need. This could allow for a real-time profile of disease presence and timely development of containment and intervention strategies, to quickly stop the spread of *Cb* in the environment and reduce human infections.

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SCRUB TYPHUS IN MEGHALAYA, INDIA: SEROPREVALENCE AND AN OUTBREAK INVESTIGATION

Sandra Albert

Indian Institute of Public Health - Shillong, Shillong, India

This paper presents the seroprevalence and risk factors associated with scrub typhus in rural Meghalaya, a state in the northeast region of India. We also report on a recent outbreak investigation in a cluster of eight villages. An age-stratified serosurvey was carried out in three districts, across 30 village clusters, including 1,328 participants. Scrub typhus seropositivity was assessed using IgG ELISA (NovaTec).Data were triangulated from a tertiary hospital, a community health centre, and two rural health centres. Epidemiological indicators, including attack rate, case fatality rate, and epidemic curve and GPS coordinates were recorded. Quantitative data were analysed using Stata, and qualitative data using Taguette. Among 1,307 participants, overall seroprevalence was 12.1%, highest in Ri Bhoi (19.9%), followed by East West Khasi Hills (8.8%) and East Khasi Hills (4.5%). At the cluster level, seroprevalence ranged from 1.6% to 43.9%, with the highest in Ri Bhoi, while two clusters in East Khasi Hills had no seropositive cases. Seroprevalence increased with age(≥60 years (30.3%)), and was higher in males (17.07%). It was also higher among animal owners (13.10% vs 8.40%) and agricultural workers (13.20% vs 10.40%). In multivariable analysis, female was associated with lower odds of seropositivity (aOR=0.53; p=0.001), while agricultural work was associated with higher odds (aOR=2.5; p<0.001). The outbreak investigation identified 59 scrub typhus cases. The attack rate was 1.48%, and case fatality rate was 1.69%. Cases were spatially clustered, with peak transmission during 12-18 November 2025. Qualitative findings indicated a partial understanding of disease transmission. Scrub typhus is locally referred to as *ñiang-soh-ot* or *ñiang-soh-stap* which can be loosely translated as disease that comes with the ripening of chestnuts.These findings show ongoing transmission and localized clustering in a biodiverse rural setting, emphasizing the need for stronger surveillance, culturally appropriate awareness, and improved peripheral health system capacity for early detection and control.

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GENETIC DETERMINANTS OF LUMEFANTRINE RESPONSE IN *PLASMODIUM FALCIPARUM*

Mary Eyi Avornyo[1], Camilla Valente Pires[2], Justin Gibbons[2], Michael Ferdig[3], John H. Adams[2]

[1]University of South Florida, Morsani College of Medicine, Tampa, FL, United States, [2]University of South Florida, College of Public Health, Tampa, FL, United States, [3]Univ of Notre Dame, Dept of Biology, Notre Dame, IN, United States

Malaria remains a major global health burden, with *Plasmodium falciparum* responsible for the majority of fatal cases. The emergence of resistance to artemisinin-based combination therapies (ACTs) threatens malaria control and underscores the urgent need for antimalarial drugs with well-defined mechanisms of action. Despite advances in drug discovery, the molecular basis of action for many antimalarials remains unclear. Lumefantrine, a key partner drug of artemisinin in frontline ACT regimens, remains essential for malaria treatment; however, its mechanism underlying emerging tolerance remains poorly understood. Reduced susceptibility to lumefantrine has increasingly been reported in African parasite populations in the absence of well-defined resistance markers, highlighting a critical gap in our understanding of parasite drug response. To address this gap, we employed a *piggyBac* mutagenesis phenotypic-genetic screen to define the genes essential for lumefantrine response in *P. falciparum*. A library of isogenic *piggyBac* mutants was exposed to a sublethal (IC_{10}) lumefantrine concentration, and relative parasite fitness was assessed during drug exposure. This analysis identified a subset of mutants exhibiting strong lumefantrine-specific sensitivity, including genes involved in RNA modification, membrane and organellar processes, and protein homeostasis. Selected individual mutants were validated using SYBR Green dose-response assays, where disruptions of genes such as PF3D7_145950 and PF3D7_0511500 make the parasite more sensitive to the drug compared with NF54. Ongoing studies are extending these findings to clinical isolates exhibiting reduced artemether-lumefantrine susceptibility. Together, this work defines molecular determinants of lumefantrine response and provides insight into pathways governing parasite survival under drug pressure.

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MEASURING RESISTANCE AND FITNESS OF CONTEMPORARY *PLASMODIUM FALCIPARUM* HAPLOTYPES ASSOCIATED WITH MALARIA CHEMOPREVENTION IN SENEGAL

Katelyn Vendrely Brenneman[1], Wesley Wong[1], Mamy Yaye Die Ndiaye[2], Stephen Schaffner[3], Bassirou Ngom[2], Imran Ullah[1], Amy Gaye[2], Djiby Sow[2], Mame Fama Ndiaye[2], Mariama Toure[2], Nogaye Gadiaga[2], Aita Sene[2], Awa Bineta Deme[2], Baba Dieye[2], Mamadou Samb Yade[2], Khadim Diongue[2], Younouss Diedhiou[2], Jules François Gomis[2], Mouhamadou Ndiaye[2], Mamadou Alpha Diallo[2], Ibrahima Mbaye Ndiaye[2], Bronwyn MacInnis[3], Dyann F. Wirth[1], Daouda Ndiaye[2], Sarah K. Volkman[1]

[1]Harvard T.H. Chan School of Public Health, Boston, MA, United States, [2]CIGASS, Dakar, Senegal, [3]Broad Institute, Cambridge, MA, United States

Whole-genome sequencing of 1879 *Plasmodium falciparum* isolates from Senegal revealed a parasite population dominated by contemporary resistance alleles: parasites carrying sensitive alleles at key resistance loci comprise 0.37% of the population, whereas resistant alleles are prevalent

(*Pfcrt* K76T 44%; *Pfmdr1* N86/Y184F 53%; *Pfdhfr* triple mutant 81%; *Pfdhps* A437G 60%). This extreme skew toward resistance-associated genotypes, in a setting with decades of chloroquine, artemisinin-based combinations, amodiaquine, and sulfadoxine-pyrimethamine use for treatment and chemoprevention, suggests that antimalarial drugs are the primary drivers of *P. falciparum* population structure in Senegal. To link these contemporary genotypes to drug response and fitness, we selected 33 clinical isolates representing 26 of the most common combined *Pfcrt, Pfmdr1, Pfdhfr,* and *Pfdhps* haplotypes (64% of all isolates), adapted them to culture, and quantified *in vitro* drug susceptibility and competitive fitness. *Pfdhfr* triple mutants were more resistant to pyrimethamine than *Pfdhfr* wild-type (WT) parasites (p<0.0001), without detectable fitness costs in the absence of drug. *Pfdhps* A437G and S436A + A437G parasites were more resistant to sulfadoxine (SDX) than WT or S436A parasites (p<0.001), confirming A437G as a key mediator of SDX resistance, but *Pfdhps* mutations did not appear to be major drivers of fitness. *Pfcrt* was a key determinant of resistance and fitness: CVIET haplotypes carrying N326S or I356T (FCB and Cam783) were more resistant to monodesethyl-amodiaquine (md-AQ) than CVIET + 220S + 271E + 371I (GB4) (p=0.001), implicating N326S and I356T in reduced AQ susceptibility. In md-AQ pooled competitive growth assays, a *Pfcrt* WT parasite was the fittest in the absence of drug, while Cam783 was the fittest in the presence of drug, consistent with being the most common mutant *Pfcrt* haplotype in Senegal (29%). These data show how long-term drug pressure has reshaped the resistance-fitness landscape in Senegal and identify contemporary haplotypes likely to drive future antimalarial resistance under evolving chemopreventive and treatment policies.

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PLASMODIUM FALCIPARUM GENE EXPRESSION DIFFERENCES AMONG SEVERE MALARIA SYNDROMES IN MALI

Emily Stucke[1], Drissa Coulibaly[2], Jonathan Lawton[3], Rafal S. Sobota[4], Jeanpaul Courneya[3], Sarika Kapadia[5], Savy M. Sebastian[1], Bryan Evan Cummings[6], Ankit Dwivedi[7], Antoine Dara[8], Abdoulaye Kassoum Kone[9], Karim Traore[10], Bouréima Guindo[11], Bourama Tangara[11], Youssouf Tolo[11], Mody Sissoko[11], Matthew B. Laurens[12], Amed Ouattara[13], Bourema Kouriba[14], Ogobara Doumbo[15], Shannon Takala Harrison[16], Kirsten E. Lyke[13], Christopher V. Plowe[1], Joana C. Silva[17], Mahamadou Ali Thera[18], Mark A. Travassos[19]

[1]University of Maryland School of Medicine, Baltimore, MD, United States, [2]Malaria Research and Training Center/USTTB, Bamako, Mali, [3]Baltimore, MD, United States, [4]Northwestern University, Chicago, IL, United States, [5]Sarika Kapadia, Baltimore, MD, United States, [6]University of Maryland, Silver Spring, MD, United States, [7]IGS, University of Maryland Baltimore, Baltimore, MD, United States, [8]USTTB, Bamako, Mali, [9]University of Bamako1 /FMOS/DEAP/MRTC, Bamako, Mali, [10]Malaria Res & Training Ctr, Bamako, Mali, [11]University of Sciences, Techniques and Technologies, Bamako, Mali, [12]Univ of Maryland Sch of Medicine, Baltimore, MD, United States, [13]Univ of Maryland School of Medicine, Baltimore, MD, United States, [14]Univ of Bamako, Bamako, Mali, [15]Malaria Research & Training Ctr, Bamako, Mali, [16]University of Maryland School of Medicine, Churchville, MD, United States, [17]Univ of Maryland School of Medicine, Silver Spring, MD, United States, [18]MRTC-P/FMOS-University of Science Techniques and Technologies of Bamako (USTTB), Bamako, Mali, [19]University of Maryland, Baltimore, MD, United States

Severe malaria disproportionately affects children under five years of age in sub-Saharan Africa, with cerebral malaria (CM) and severe malarial anemia (SMA) representing clinically distinct syndromes responsible for the highest mortality. Although host responses contributing to these syndromes have been described, less is known about whether *Plasmodium falciparum* exhibits syndrome-specific transcriptional variation. We conducted a severe malaria study in Mali, West Africa from 2014-2018 to directly compare parasite gene expression among severe malaria syndromes and identify syndrome-specific transcriptional patterns. We analyzed *P. falciparum* transcriptomes from children with severe malaria using RNA sequencing. Severe malaria cases included CM (n = 25), SMA (n = 38), and concurrent CM + SMA (n = 12). Principal component analysis of all expressed genes showed substantial overlap among severe malaria syndromes, with no distinct clustering observed, revealing the absence of broad, distinct transcriptional profiles among severe malaria syndromes. Differential gene expression (DGE) was assessed using a quasi-likelihood F-test, and we corrected for multiple comparisons using a false discovery rate threshold of < 0.05. Significant gene expression differences were identified between CM and SMA, CM and CM + SMA, and SMA and CM + SMA. Minimal overlap in DGE was noted across these comparisons. Despite substantial overlap in global gene expression patterns, pairwise analyses revealed syndrome-associated *P. falciparum* transcriptional differences, suggesting that parasite gene expression differences may contribute to clinical phenotypic variation. Syndrome-specific patterns, particularly those associated with SMA and concurrent CM + SMA, suggest parasite biological variation beyond host-driven effects. These findings provide insights into parasite mechanisms that may be associated with the development of distinct severe malaria syndromes and inform future studies of pathogenesis and targeted interventions.

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PROGRESS AND OPPORTUNITIES IN DISTINGUISHING RECRUDESCENCE FROM REINFECTION IN RECURRENT *PLASMODIUM FALCIPARUM* INFECTIONS

Megan Gelement[1], George Tollefson[2], Ozkan Aydemir[3], Mwiche Siame[4], Philip Thuma[5], William J. Moss[6], Jonathan J. Juliano[7], Jeffrey A. Bailey[2], Joel S. Bader[1], Matthew M. Ippolito[8]

[1]Johns Hopkins University, Baltimore, MD, United States, [2]Brown University, Providence, RI, United States, [3]Univ of Massachusetts Medical School, Worcester, MA, United States, [4]Macha Research Trust, Choma, Zambia, [5]Macha Research Trust, Mechanicsburg, PA, United States, [6]Johns Hopkins Univ, Lutherville, MD, United States, [7]Univ of North Carolina, Chapel Hill, NC, United States, [8]Johns Hopkins University School of Medicine, Baltimore, MD, United States

The prevalence of polyclonal *Plasmodium falciparum* infections, particularly in high-transmission areas, can make it difficult to identify the source of recurrent infection in samples collected from the same patient at two or more time points. Recurrent infections may be recrudescent (arising from one or more strains not cleared after initial infection) or may arise from new strains in a subsequent independent infection. The distinction between recrudescence and reinfection is clinically relevant for assessing and adjusting therapeutic response and for identifying genetic sources of drug resistance. Molecular inversion probe (MIP) panels are one method used in clinical trials and therapeutic efficacy studies to measure alleles present across the *P. falciparum* genome with higher resolution than amplicon panels and without the expense or alignment ambiguity of whole genome sequencing (WGS). Index and recurrent samples from 13 study participants with recurrent malaria, including 2 pairs of known identical infections sampled 1 week apart, were genotyped using a 1,783-loci MIP panel in addition to standard methods. We compared multiple methods for recurrence classification. Conventional *msp1* and *msp2* genotyping resulted in 4 indeterminate results, 3 of which were resolved with the addition of *ama1* amplicon data. MIP data analyzed using the asterTES R package resolved all infections, including the known identical infections, and identified a shared subset of polygenomic infection between two individuals corroborated by separate *ama1* amplicon data. Investigations of read depth and library coverage suggest pathogen density to be an informative covariate for future models. We also discuss computational generation of MIP-type data from WGS repositories. This work establishes a baseline for further investigation of recurrent *P. falciparum* infection classification in MIP data.

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GENOMIC EVIDENCE OF CHANGING TRANSMISSION DYNAMICS AND POPULATION STRUCTURE OF *PLASMODIUM VIVAX* DURING MALARIA ELIMINATION IN THE GREATER MEKONG SUBREGION

Emma Rowley[1], Bing Guo[1], Mariusz Wojnarski[2], Michele D. Spring[3], Sabaithip Sriwichai[2], Brian A. Vesely[2], Joana C. Silva[4], Norman C. Waters[2], Somethy Sok[2], Satharath Prom[5], Sidhartha Chaudhury[2], Chanthap Lon[6], Dysoley Lek[7], David Saunders[8], Kathleen Stewart[9], Timothy D. O'Connor[4], Shannon Takala-Harrison[1]

[1]Center for Vaccine Development and Global Health, University of Maryland School of Medicine, Baltimore, MD, United States, [2]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [3]State University of New York, Upstate Medical University, Dewit, NY, United States, [4]Institute for Genome Sciences, University of Maryland School of Medicine, Baltimore, MD, United States, [5]Royal Cambodian Armed Forces, Phnom Penh, Cambodia, [6]International Center of Excellence in Research & Laboratory of Malaria and Vector Research, National Institutes of Health, Phnom Penh, Cambodia, [7]National Center for Parasitology, Entomology, and Malaria Control, Phnom Penh, Cambodia, [8]US Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD, United States, [9]Center for Geospatial Information Science, University of Maryland, College Park, MD, United States

Estimates of *Plasmodium vivax* population demography can reveal changes in transmission dynamics following antimalarial interventions to inform resource prioritization. Here, we analyzed whole-genome sequence data from 684 *P. vivax* isolates to estimate parasite relatedness, population structure, and migration patterns over time using identity-by-descent (IBD), a measure of genetic similarity. Isolates were collected in Cambodia and neighboring provinces in Thailand and Vietnam from 2009 to 2022. Average pairwise IBD sharing among isolates from Cambodia and Thailand increased from 0.65% in 2009 to 5.7% in 2022, consistent with reduced transmission. Following interruptions to the Village Malaria Worker program and increased cases in 2016-2018, IBD declined sharply from 4.2% in 2018 to 1.4% in 2019 ($p = 2.5e-09$), suggesting IBD estimates may capture disruptions in control efforts. After 2019, IBD rebounded quickly, likely reflecting Cambodia's intensification of elimination efforts in transmission hotspots. IBD increased significantly in Oddar Meanchey and Kratie provinces over time, but not in Stung Treng ($p = 2.6e-22$, 0.0008, and 0.08, respectively). However, IBD network density, the proportion of related isolate pairs, increased in all three provinces over time. These results suggest that metrics such as network density may capture changes in transmission dynamics missed by summary relatedness measures. Admixture analysis identified a distinct statistical subpopulation from Stung Treng, potentially reflecting cross-border transmission with Laos. Combined IBD and admixture patterns suggested parasite movement into Kratie, consistent with a role as a transmission sink. Migration and population size surfaces (MAPS) identified significant migration barriers (>90% posterior probability), corresponding to parts of the Mekong River and the Thai Cambodian border marked by a steep escarpment. These results demonstrate that population genomics can detect the impact of intervention disruptions, reveal fine-scale transmission dynamics, and identify potential barriers relevant to targeted elimination strategies.

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EVOLUTION OF A CLONALLY EXPANDING *PLASMODIUM FALCIPARUM KELCH13*-R561H LINEAGE IN EASTERN MYANMAR

Xue Li[1], Grace A. Arya[1], Shalini Nair[1], Francis Nkemngo[1], Aung Myint Thu[2], Aung Pyae Phyo[3], Mallika Imwong[4], François Nosten[2], Timothy Anderson[1]

[1]Texas Biomedical Research Institute, San Antonio, TX, United States, [2]Shoklo Malaria Research Unit, Mae Sot, Thailand, [3]Mahidol Oxford Tropical Medicine Research Unit, Yangon, Myanmar, [4]Mahidol University, Bangkok, Thailand

Clonal transmission of *Plasmodium falciparum* has been widely observed in Southeast Asia and South America and is increasingly reported in parts of Africa. Such clonal expansions facilitate the spread of linked adaptive mutations, including those associated with antimalarial drug resistance. Here, we investigate the genomic dynamics of a clonally expanding parasite lineage carrying the *Kelch13-R561H* mutation that has dominated malaria transmission in eastern Myanmar. To characterize the evolutionary trajectory of this lineage, we generated high-quality genome assemblies from 13 *Kelch13*-R561H parasite clones using a combination of Nanopore and PacBio long-read sequencing. These assemblies were analyzed together with more than 300 longitudinally collected *P. falciparum* isolates sequenced with Illumina technology between 2015 and 2025 that belong to the *Kelch13*-R561H transmission cluster. Using this combined dataset, we examined the accumulation of single nucleotide polymorphisms (SNPs), indels and large structural variants during the decade-long clonal expansion of this lineage. We identified *de novo* mutations and estimated the mutation rate within this field-derived clonal lineage. Structural variant analyses identified several lineage-specific rearrangements, highlighting the utility of long-read sequencing for resolving complex genomic changes during parasite transmission. Notably, we detected the emergence of *hrp2* gene deletions within the *Kelch13*-R561H cluster rising to 87% frequency in 2024. These findings demonstrate the genomic stability yet adaptive potential of a clonally expanding *P. falciparum* lineage and highlight the importance of integrating long-read sequencing with longitudinal population genomics to monitor parasite evolution. The recent emergence of *hrp2* deletions within this dominant lineage raises concerns for the continued effectiveness of HRP2-based rapid diagnostic tests and underscores the need for genomic surveillance in regions with clonal malaria transmission.

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TEMPORAL MOLECULAR SURVEILLANCE AND WHOLE-GENOME ANALYSIS OF *PLASMODIUM FALCIPARUM* REVEAL STABLE POPULATION STRUCTURE AND TRENDS IN ANTIMALARIAL RESISTANCE MARKERS IN CAMEROON

Vincent N. Ntui-Njock[1], Brandon N. Amambua-Ngwa[1], Martha A. Demba[1], Magdany Fru[2], Nora N. Nganyewo[1], Livinus N. Tangi[2], Pilate N. Kwi[1], Hanesh Chi[2], Oriero C. Eniyou[1], Vincent PK Titanji[2], Tobias O. Apinjoh[2], Alfred Amambua-Ngwa[1]

[1]Medical Research Council Unit The Gambia at London School of Hygiene and Tropical Medicine, Banjul, 0000, Gambia, [2]University of Buea, Southwest, Cameroon, Banjul, 0000, Gambia

Advances in malaria genomics including temporal molecular surveillance and whole genome sequencing provides powerful tool to track parasite infection complexity, genetic diversity, population structure and antimalarial resistance markers. In this study we leverage these approaches to investigate temporal-spatial trends in infection complexity and key antimalarial resistance markers in Cameroon. This study included 10,207 participants from cross-sectional survey between 2019-2022 and 2025 alongside whole genome analysis of *Plasmodium falciparum* sequences from 2013, 2016/2017. Malaria infection was accessed using blood malaria rapid diagnostic test (mRDT) and real-time polymerase-chain-reaction (qPCR)-VarATS. Amplicon sequencing of 992 *P. falciparum* isolates was performed and analyses done in R. Among the 10,207 individuals screened, prevalence by mRDT was 23.67% in 2019-2022 and 26.3% in 2025

with 58.77% and 50.24%, respectively by qPCR-VarATS. *P. falciparum* drug resistance analysis shows persistent high frequencies of antifolate resistance markers. Sulfadoxine-pyrimethamine quadruple mutants (IRNI-SGKAA) predominated (33.2%), with lower frequencies of quintuple (IRNI-SGKGS) 9.4% and sextuple (IRNI-AGKGS) 3.8%, haplotypes. Increasing prevalence in *Pfcrt*-CVMNK ranged 78.8-81.3% and declining CVIET declining from 21.2-18.7% were observed. *Pfmdr1*-NFD haplotype increased from 36.7% to 58.0%, with no WHO-validated pfk13 mutations detected. Genome-wide analysis shows overall low genetic differentiation (mean FST=0.049) of *Plasmodium falciparum* population between 2013 and 2016/2017. However, several loci displayed high FST up to 0.67 indicating localised selection across multiple chromosomes. Within-host diversity (Fws) values were high (median >0.9) indicating a predominantly clonal infections with 2013 showing a slightly greater diversity with more mixed infections. There is persistent antifolate resistance, declining *Pfcrt*-resistant haplotypes, and increasing *Pfmdr1* NFD frequencies while parasite population structure remained stable with localised genomic differentiation.

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*VAR*CODING RESOLVES *PLASMODIUM FALCIPARUM* ANTIGENIC DIVERSITY BETWEEN DIFFERENT TRANSMISSION SETTINGS IN GHANA

Lucindah N. Fry-Nartey[1], Michaella Ahorbo[1], Samuel Nyarko[2], Nukunu E. Akyea-Bobi[1], Prince Z. Horlortu[1], Henrietta Sasu[1], Nigel Dolling[1], Rita A. Boateng[1], Benedicta Mensah[1], Kathryn E. Tiedje[3], Anita Ghansah[1]

[1]Noguchi Memorial Institute for Medical Research, University of Ghana, Accra, Ghana, [2]Department of Cellular Biology, University of Georgia, Athens, Georgia, United States, Athens, GA, United States, [3]Department of Microbiology and Immunology, Bio21 Institute and The Peter Doherty Institute for Infection and Immunity, The University of Melbourne, Melbourne, Australia, Melbourne, Australia

The *var* multigene family encodes PfEMP1, the major variant surface antigen of the *Plasmodium falciparum* blood stages and a key target of naturally acquired immunity. Thus, *var* gene diversity reflects the antigenic landscape encountered by human hosts and variation in parasite populations that shape immune recognition. However, current molecular surveillance methods exclude *var* genes due to challenges in assembling their highly diverse sequences. As a result, these approaches capture patterns of parasite relatedness but miss antigenic diversity and immune-relevant population structure, limiting insight into how parasite populations interact with host immunity. This study fills this gap by assessing the utility of *var*coding, a genetic fingerprinting method targeting a ~450 bp region within the DBLα domain of *var* genes, as a complementary tool for malaria surveillance. We applied this method to dried blood spot samples from 208 children (< 15 years) with uncomplicated malaria from two ecologically distinct sites in Ghana: Cape Coast (moderate – low transmission) and Begoro (high transmission). Deep sequencing revealed high DBLα type diversity in both Cape Coast (2,591 types) and Begoro (6,522 types), with minimal spatial overlap (11% shared), indicating highly localized antigenic repertoires. Temporal persistence of DBLα types was low (8.7% in Cape Coast; 3.0% in Begoro), indicating rapid turnover of antigenic variants. Multiclonal infections were predominant in both sites and increased over time in Begoro (63.9% to 89.7%), while being stable in Cape Coast (76.2% to 76.5%). Across all time points, DBLα isolate repertoire similarity was low within and between sites, indicating extensive parasite diversity. Our findings show that *var*coding captures dimensions of population structure and antigenic diversity not resolved by conventional surveillance methods. Incorporating *var* gene information into surveillance frameworks can enhance our understanding of population immunity, reveal hidden structure in transmission dynamics and improve the interpretation of how *P. falciparum* populations respond to control interventions.

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PLAMODIUM FALCIPARUM CONNECTIVITY AND EVOLUTION IN THE DEMOCRATIC REPUBLIC OF THE CONGO

Ronald Futila Kyong-shin[1], Christopher Michael Hennelly[1], Reagan Luvande Okingo[2], Aliocha Nkodila[2], Andre Nsana[2], Adrien Nsiala[2], Linda James[1], Jacob Matthew Sadler[1], Abebe Alemu Fola[3], Karamoko Niare[3], Jeffrey A. Bailey[3], Melchior Mwandagalirwa Kashamuka[4], Antoinette Tshefu Kitoto[4], Jonathan J. Juliano[1], Joris Likwela[2], Dieudonné Mumba[5], Parul Johri[1], Jonathan Parr[1]

[1]University of North Carolina at Chapel Hill, Chapel Hill, NC, United States, [2]Sante Rurale (SANRU) Asbl, Kinshasa, Democratic Republic of the Congo, [3]Brown University, Providence, RI, United States, [4]Kinshasa School of Public Health, Kinshasa, Democratic Republic of the Congo, [5]National Institute for Biomedical Research, Kinshasa, Democratic Republic of the Congo

The Democratic Republic of the Congo (DRC) is a nexus linking West and East African *Plasmodium falciparum* parasite populations, yet to-date, whole-genome sequencing (WGS) analysis has been restricted to the capital (Kinshasa). Previous molecular inversion probe and amplicon sequencing studies showed a largely panmictic population with isolation-by-distance influenced by geographic barriers like the Congo River. Here, we used nationwide whole-genome data to assess parasite connectivity and gene flow. The PaluSeq project, establishing malaria molecular surveillance in DRC, is actively enrolling participants at 36 sites across nine provinces covering major DRC regions. Enrolled participants complete a questionnaire and provide dried blood spot samples. We chose 200 representative monoclonal and dominant-clone samples from 9 provinces. We also included 219 previously existing WGS samples. Overall, 214 WGS samples passed quality control and filtering after variant calling using GATK4 MalariaGen best practices. Restricting to the core genome and synonymous sites, we computed genetic diversity metrics and pairwise F_{ST}. Population structure was assessed with STRUCTURE, spatial patterns of gene flow were characterized using the Estimation of Effective Migration Surfaces (EEMS), and demographic modeling with DaDi. While STRUCTURE confirms that the DRC population is a single panmictic population, EEMS results reveal distinct spatial patterns of gene flow across the DRC: one high-connectivity region in the West and Northwest, and two reduced-connectivity regions in the Center and East. The high-connectivity western region covers the navigable portion of the Congo River, the country's main waterway. This spatial structure is corroborated by pairwise F_{ST} values ranging from 0.02 to 0.08, with moderate differentiation ($F_{ST} \geq 0.05$) consistently involving provinces within regions of reduced gene flow zones by EEMS. Initial demographic modeling reveals strong asymmetric East-to-West gene flow, with the East acting as the main parasite source and the Center showing minimal outward migration, behaving as a demographic sink.

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THE SEROTONIN RECEPTOR 5'HTR2C REGULATES ALVEOLAR MACROPHAGE RESPONSES POST-HELMINTH INFECTION

Marissa Schroeter[1], Arman Sawhney[1], John J. Ponessa[2], Krupa Chavan[1], Juliana C. Corrêa-Velloso[3], Leandro C. Clementino[3], Jianya Peng[2], Andrew P. Thomas[2], Mark C. Siracusa[4]

[1]Center for Immunity and Inflammation, Rutgers, The State University of New Jersey, Newark, NJ, United States, [2]NemaGen Discoveries, Princeton, NJ, United States, [3]Department of Pharmacology, Physiology, and Neuroscience, Rutgers. The State University of New Jersey, Newark, NJ, United States, [4]Center for Immunity and Inflammation, Rutgers, The State University of New Jersey, Newark, NJ, United States

Alveolar macrophages (AMs)are highly specialized cells that have long been recognized for their ability to regulate lung homeostasis. Additionally, it is well appreciated that alveolar macrophages perform important antihelminth functions and are required to promote wound healing responses following the passage of larvae through the lung. Despite these advances, the

molecular features of AMs that allow them to perform these tissue-specific functions remain to be fully understood. Recent RNAseq data has revealed that AMs uniquely express the serotonin receptor 5'HTR2c, the only immune cell population to do so. Knowing that serotonin is released at the site of injury by activated platelets, we hypothesized that 5'HTR2c regulates the activation of AMs in the context of helminth-induced damage. To test this, we employed the hookworm *Nippostrongylus brasiliensis* (Nb), that promotes severe lung pathology and the robust activation of alveolar macrophages. Following infection, we observed that mice lacking expression of5'HTR2c on AMs (HTR2c flox x CD11c-Cre mice) failed to properly resolve Nb induced-damage as highlighted by an increased accumulation of red blood cells in the bronchoalveolar spaces of the lungs. When investigating the mechanisms dictating these phenotypes, we identified that serotonin is a potent initiator of robust calcium responses in AMs measured by using the calcium indicator dyeFura2. Further, we determined that serotonin-induced calcium signals were 5'HTR2c-dependent, despite the fact that AMs also express the 5'HTR2b receptor. We performed an RNAseq analysis of HTR2c-deficient AMs and found them to lack the expression of molecules associated with controlling lung hemorrhaging and maintaining lung homeostasis, suggesting that AMs may regulate vascular permeability and the mitigation of tissue damage following a helminth infection. Collectively, these data suggest that the selective expression of the 5'HTR2c receptor by AMs allow them to properly clear debris and restore lung homeostasis following helminth-induced injury. Our ongoing studies are working to better understand the molecular mechanism dictating these changes and the long-term effects of targeting this pathway in the context of helminth-induced inflammation.

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SINGLE-CELL ATLAS OF THE *SCHISTOSOMA MANSONI* CERCARIA: A NEW TALE OF A NEW TAIL

Yuxuan Xu[1], Sarah Kerr[1], Teresa Attenborough[2], Frances Blow[2], Gabriel Rinaldi[3], Matthew Berriman[2], Kate Rawlinson[1]

[1]Marine Biological Laboratory, Woods Hole, MA, United States, [2]University of Glasgow, Glasgow, United Kingdom, [3]University of Oxford, Oxford, United Kingdom

Schistosoma mansoni is a parasitic flatworm that causes schistosomiasis, a neglected tropical disease affecting an estimated 2% of the global population. We generated a single-cell RNA-seq whole-body atlas of the cercaria, the human-invading larval stage, identifying 24 cell types, 22 of which we spatially validated using HCR *in situ* hybridization. This revealed cell type heterogeneity between body and tail not previously apparent morphologically, including a novel, immunogenic tail tegument cell type. Using phylostratigraphy, we found that tail-specific cell types tend to express more recently evolved genes than their body-specific counterparts, providing insight into the evolutionary origin of the cercarial tail. Together, our morphological and transcriptomic characterization supports the tail being an evolutionarily novel organ and suggests it has a dual locomotory and immunogenic role.

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A GENOME WIDE ANTIGEN-ANTIBODY SCREEN IDENTIFIES A YEAST-BASED THERAPEUTIC VACCINE CANDIDATE FOR CHAGAS DISEASE

Mira Loock, Lissa Cruz-Saavedra, Valeria Bernal Araujo, Igor Cestari

McGill University, Ste. Anne-de-Bellevue, QC, Canada

Chagas disease is caused by *Trypanosoma cruzi* infection and results ind ecades-long, chronic, debilitating, and lethal disease. There is no vaccine available, largely due to the lack of vaccine targets. We generated a *T.cruzi* genome-wide library for yeast surface display and screened it with antibodies from Chagas disease patients to identify antigen-antibody interactions. We identified hundreds of *T. cruzi* antigens that are immunogenic in humans and mapped their antibody-binding sites at nucleotide resolution. Hundreds of immunogens were conserved across strains, divergent from human proteins, and expressed across all *T. cruzi* infectious forms, revealing potential vaccine targets. We vaccinated mice with a 63-amino-acid-long highly immunogenic region using recombinant protein (rIR1) or yeast surface expression (yIR1). yIR1 surpassed rIR1, reducing parasitemia in blood and tissues in a prophylactic, acute-stage vaccination model, validating IR1 as an immunogen and yeast as a vaccine vehicle for Chagas disease. Notably, therapeutic vaccination of chronically infected mice with yIR1 cleared parasites from muscle tissues and was accompanied by the production of α-IR1 antibodies and an increased proportion of CD8+ T cells, indicating that a vaccine for treating chronic Chagas disease may be feasible. The antigen-antibody screen revealed hundreds of immunogens for vaccine, diagnostic, and biomarker discovery, and uncovered yIR1 as a therapeutic vaccine candidate for Chagas disease.

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THE BITES OF NON-INFECTED SAND FLIES PROMOTE THE RE-ACTIVATION OF L. MAJOR-CAUSED CUTANEOUS LEISHMANIASIS, ULTIMATELY IMPROVING THE COMPETENCE OF BOTH ANIMAL RESERVOIRS AND SAND FLY VECTORS

Pedro Amado Cecilio[1], Maria Disotuar[2], Eva Iniguez[2], Luana Rogerio[2], Kristina Tang[2], Luiza dos Santos Felix[2], Johannes Doehl[2], Tiago D. Serafim[2], Claudio Meneses[2], Caroline Percopo[3], Jesus G. Valenzuela[2], Fabiano Oliveira[2]

[A]Vector Biology Section, Laboratory of Malaria and Vector Research, [1]Vector Molecular Biology Section, Laboratory of Malaria and Vector Research, NIAID, National Institutes of Health, Rockville, MD, United States, [2]Flow Cytometry Core, Laboratory of Malaria and Vector Research, NIAID, National Institutes of Health, Rockville, MD, United States

Most sand fly-transmitted *Leishmania* species pathogenic to humans are zoonotic agents, meaning that animal reservoirs play an essential lepidemiological role in leishmaniasis. However, our understanding of such animal reservoirs, and their interactions with the sand fly vectors is still limited. Here, we took advantage of different cutaneous leishmaniasis (CL) models to try to understand whether the bites of non-infected sand flies impact infection/disease. When we subjected active CL lesions, in both BALB/c andC57BL/6 mice, to a single event of non-infected sand fly bites, no impact on disease progression was observed. However, when we exposed healed CL lesions in the ears of C57BL/6 mice to a single event of non-infected sand fly bites, a transient pathological exacerbation was observed. Pathological changes of greater magnitude were observed in the context of multiple exposure events to the bites of non-infected sand flies, phenotype that was reproduced by the injection of sand fly saliva into healed CL lesions. Importantly, such changes were accompanied by a significant increase in the ear parasite burden of exposed versus control animals. Mechanistically, known chemoattractants in the sand fly saliva, injected into the skin during feeding, induce massive neutrophil recruitment to the bite site, which in turn promotes a change in the inflammatory milieu and the generation of granzyme B-producing "pathogenic"CD8+ T cells. Such an inflammatory phenotype makes, in turn, these sand fly bites-exposed mice more attractive to subsequent sand fly bites. Aligned with this notion, alterations in sand fly infection quality were also observed; sandflies recovered after a third, and second exposure events allowed a better development of infection (with proportionality) than those recovered after the first exposure event. Overall, these results suggest that sand fly vectors and animal reservoirs enhance each other's competence, ensuring the perpetuation of *Leishmania* parasites in the wild.

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UNCOVERING THE ROLE OF A *CRYPTOSPORIDIUM PARVUM* THROMBOSPONDIN PROTEIN IN MACROGAMONT DEVELOPMENT

Zoe Reynolds, Sumiti Vinayak Alam

Department of Pathobiology, College of Veterinary Medicine, University of Illinois at Urbana-Champaign, Urbana, IL, United States

The protozoan parasite *Cryptosporidium parvum* is a global cause of diarrheal illness, primarily affecting calves, immunocompromised people, and children. No drug or vaccine is available to treat or prevent cryptosporidiosis and/or reduce disease transmission. Ingested oocysts excyst sporozoites that invade the host's intestinal epithelial cells. Post-invasion, the parasite replicates asexually and then proceeds to a sexual cycle, forming male (microgamonts) and female (macrogamonts) gamonts. Male gametes released from the macrogamont fertilize the macrogamonts, resulting in oocysts. The molecular mechanisms underlying macrogamont maturation and signaling interactions between gametes leading to fertilization are not understood. We identified a *C. parvum* thrombospondin repeat-containing protein (CpTSP2) with a Lin-12 repeat/Notch signaling domain predicted by SMART protein domain prediction tool. Since Lin-12/Notch signaling proteins are known to mediate development and cell-cell interaction, we hypothesize that CpTSP2 may be involved in the maturation of the macrogamont and communication with the male gametes for fusion. To determine the localization of CpTSP2 during the parasite's developmental cycle, we generated a stable transgenic *C. parvum* line with a hemagglutinin tag appended to the C-terminus of the protein using CRISPR/Cas9 editing and an immunocompromised mouse infection model (n= 2 cages of 4). Using super-resolution and ultrastructure expansion microscopy, we found that CpTSP2 localizes to macrogamont, oocyst, and asexual stages. All immunofluorescence assays and expansion microscopy were repeated twice, images shown are representative images. Our imaging analysis reveals that macrogamonts of various sizes express CpTSP2 differently. While some macrogamonts show an intracellular localization with the protein in the cytoplasm, others exhibit punctate expression of CpTSP2 near the membrane. These variations in CpTSP2 expression at different stages of macrogamont development suggest a role in gamont maturation and/or fertilization. Our plans include using a pharmacological agent to inhibit CpTSP2 function, delete the *tsp2* gene, and determine the effect on macrogamont maturation and fertilization. Understanding the mechanisms controlling macrogamont development and fertilization for oocyst production will pave the way for developing future strategies to reduce disease transmission.

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ESTABLISHMENT OF A TRANSFECTION SYSTEM IN *NAEGLERIA FOWLERI*

Caroline M. Palmentiero Stephan, Jillian E. m. McKeon, Colm P. Roster, James C. Morris

Eukaryotic Pathogens Innovation Center, Department of Genetics and Biochemistry, Clemson University, Clemson, SC, United States

The pathogenic free-living amoeba Naegleria fowleri is the causative agent of primary amoebic meningoencephalitis (PAM), a rapidly progressing infection with a mortality rate exceeding 97%. The development of effective therapeutics has been hindered by the lack of molecular tools to study gene function and validate potential targets. Here, we report progress toward establishing heritable transgene expression systems in N. fowleri. Through an iterative process, we optimized vector design and transfection conditions to enable generation of transgenic amoebae. Our vector encodes the selectable marker puromycin N-acetyltransferase (pac), codon optimized for N. fowleri, linked to the fluorescent protein mCherry through a self-cleaving T2A peptide. Expression is driven by the 5′ and 3′ UTRs of a conserved N. fowleri actin gene (NfTy_067520, AmoebaDB). For transfection, we found that 40 kDa polyethylenimine (PEI-40) effectively mediated plasmid uptake and transgene expression. Following puromycin selection, we generated stable antibiotic-resistant, fluorescence-expressing lines in > 90% of cells. To expand the utility of this system, we demonstrated subcellular targeting of mCherry to peroxisomes through the addition of a peroxisomal targeting sequence (PTS1). We also validated a second selectable marker, blasticidin-S deaminase (bla), codon optimized to N. fowleri. Finally, fusion of a trimethoprimregulated destabilizing domain to mCherry did not disrupt reporter expression, supporting its future use as a tool for conditional regulation of protein expression in N. fowleri. Together, these advances establish a foundation for genetic manipulation in N. fowleri and provide new tools for investigating parasite biology and validating therapeutic targets.

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A SNARE-LIKE PLASMODIUM RHOPTRY NECK PROTEIN IS REQUIRED FOR SEALING OF THE PARASITOPHOROUS VACUOLE DURING MEROZOITE INVASION

Grace Vick, Annabella Castiglione, Carrie F. Brooks, Vincent Starai, Vasant Muralidharan

University of Georgia, Athens, GA, United States

Plasmodium falciparum, the causative agent of human malaria, are obligate intracellular parasites during all replicative stages of their complex lifecycle. Critical for their successful expansion is their ability to form and replicate inside avacuole derived from the host RBC membrane during invasion called the parasitophorous vacuole (PV). During invasion, the merozoite propels itself inside the host cell, bringing the RBC membrane around itself, which forms the nascent PV. But surprisingly, we do not know how the RBC membrane is sealed during merozoite invasion to form the PV. Our work has identified a conserved membrane protein (Pf3D7_1117400) that has distant homology to vesicle-SNARE proteins. Using CRISPR/Cas9 genetic engineering to create a conditional knockdown of Pf3D7_1117400, we determined that it localizes to the rhoptry neck. Hence, we termed this protein, SNARE-like rhoptry neck protein (SNARON). Knockdown of SNARON led to parasite death after the first asexual cycle, indicating that SNARON is essential for intraerythrocytic development. We observe SNARON knockdown parasites egress normally, but fail to form rings. Using live video microscopy, we find that SNARON knockdown merozoites are unable internalize but do induce significantly faster echinocytosis in host cells. Additionally, the few merozoites that manage to successfully internalize fail to form a PV at all. When SNARON is expressed in *Saccharomyces cerevisiae,* we find that vacuolar membranes form defective structures, indicating a role for SNARON in membrane fusion. Together, these data suggest that SNARON works at the site of merozoite invasion to drive sealing of the PV from the RBC membrane.

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STRUCTURAL BASIS FOR HOST MEMBRANE BINDING AND REMODELING BY THE MALARIAL MOVING JUNCTION

Meseret T. Haile[1], Daphne A. Kaxiras[2], James Zhen[1], Carolyn L. Lee[1], Britney Jiang[3], Jennifer L. Small-Saunders[3], Chi-Min Ho[1]

[1]Department of Microbiology and Immunology, Columbia University Irving Medical Center, New York, NY, United States, [2]MD-PhD Program, Columbia University Vagelos College of Physicians and Surgeons, New York, NY, United States, [3]Division of Infectious Diseases, Department of Medicine, Columbia University Irving Medical Center, New York, NY, United States

The malaria-causing parasite, *Plasmodium falciparum (Pf),* invades human redblood cells (RBCs) through a rapid process that begins when merozoites – an invasive life cycle stage - attach to the host cell surface. A defining step in invasion is the formation of a ring-shaped moving junction, which staples the merozoite and RBC membranes together, forming a "portal" that the parasite pushes its way through. The core of the moving junction is formed by the interaction between *P. falciparum* Apical Membrane Antigen 1 (PfAMA1), displayed on the merozoite surface, and Pf Rhoptry Neck Protein 2 (PfRON2), which the parasite secretes into the RBC membrane. While prior structural studies have characterized fragments of this interaction, the full architecture of the moving junction in its native context and its function during invasion remain unresolved. To address this, we isolated the natively assembled endogenous moving junction by introducing a C-terminal

epitope tag into PfRON2 then used Percoll synchronization, together with Compound 1 and Cytochalasin-D to enrich invasion-stalled merozoites. We then affinity purified PfRON2 along with other bound components of the moving junction from the stalled merozoites and used single particle cryoEMto determine the structure of the intact complex. Our structure provides clues that the moving junction may play an active role during invasion, beyond simply tethering the parasite to the RBC. Together, our findings define the architecture of the endogenous moving junction, provide new insight into its role during invasion, and offer a structural framework for rational, structure-guided design of therapies.

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PERSISTENT GROSS NEUROLOGIC DEFICITS AFTER PEDIATRIC CEREBRAL MALARIA ARE ASSOCIATED WITH CEREBROSPINAL FLUID MARKERS OF NEURONAL INJURY AND METABOLIC ALTERATIONS

Alejandro A. Soto[1], Kagan A. Mellencamp[1], Proveen Kothagundla[1], Vijay KR Bommireddy[1], Sai Meghana Imadabattini[1], Michael G. Goings[1], Ruth Namazzi[2], Robert Opika Opoka[2], Thomas M. O'Connell[1], Richard Idro[2], Chandy C. John[1], **Dibyadyuti Datta**[1]

[1]Indiana University School of Medicine, Indianapolis, IN, United States, [2]Makerere University College of Health Sciences, Kampala, Uganda

Cerebral malaria (CM) is a major cause of acquired neurodisability in children. Gross neurologic deficits occur in 25-40% of survivors at discharge and persist in a subset, but the CNS processes underlying poor recovery are not well defined. We performed a secondary analysis of a prospective cohort of Ugandan children aged 6 months to 12 years with CM. Neurologic exams were conducted at discharge and 6 months. Admission clinical features, blood and cerebrospinal fluid (CSF) biomarkers, and untargeted CSF metabolomics were compared by 6-month neurologic outcome. Among 233 survivors, 82 (35%) had deficits at discharge; 11 (5%) had persistent deficits at 6 months, most commonly motor and cranial nerve abnormalities. Children with persistent deficits had lower home environment z-scores (P=0.002), deeper coma (median Blantyre Coma Score 1 vs. 2; P<0.001), and more EEG seizures (27% vs. 5%; P=0.004). Admission markers of metabolic and neuronal injury were higher in children with deficits, including lactate dehydrogenase (P=0.021), uric acid (P=0.004), and total tau in CSF (P=0.011) and plasma (P=0.016). CSF metabolomics (available in 117 children without vs. 6 with deficits) showed partial separation by outcome and identified 36 differentially abundant metabolites (≥1.25-fold change, P≤0.01), including metabolites involved in tryptophan, nucleotide, lipid, and energy metabolism, as well as exogenous compounds consistent with drug and environmental exposures. Persistent neurologic deficits in CM are associated with acute neuronal injury, metabolic stress, and distinct CSF metabolic profiles. These findings highlight CNS pathways associated with poor neurologic recovery and provide a foundation for future mechanistic studies of the neuropathogenesis of pediatric CM.

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A FINE SCALE SINGLE-CELL ROADMAP OF THE CELL CYCLE AND METACYCLOGENESIS IN *TRYPANOSOMA CRUZI*

Caroline Keroack[1], Eric Salomaki[2], Sophie Marcus-Wade[1], Jacqueline Lopez[1], Madeline Malfara[1], Erin Kennedy[1], Christopher de Graffenried[1]

[1]Department of Molecular Microbiology and Immunology, Brown University, Providence, RI, United States, [2]Center for Computational Biology of Human Disease, Brown University, Providence, RI, United States

Chagas disease, caused by the protozoan parasite *Trypanosoma cruzi*, remains a significant global health burden with millions of people at risk of infection. The parasite undergoes several morphological changes during its life cycle allowing it to adapt to different host environments. Despite advances in transcriptomics, much remains unknown about the molecular mediators underlying key processes in epimastigotes, the insect stage of the parasite. Single-cell RNA sequencing has been used to define stage-specific gene expression in *T. cruzi,* including a recently generated atlas of the major life cycle stages. However, these data have not resolved the epimastigote cell cycle to the phase level. Here, we present a high-depth scRNA-seq data set that resolves both the cell cycle and spontaneous metacyclogenesis. To confidently distinguish cell cycle from metacyclic clusters, we employed two growth conditions: one that enriched for cells undergoing metacyclogenesis and a second that favors proliferation. We identified markers of each cell cycle phase and validated their temporal expression by immunofluorescence microscopy. We also identified candidates for each stage of metacyclogenesis and have validated a previously uncharacterized RNA-binding protein as a marker of the first intermediate stage. Additionally, we have used the Short Hammerhead Aptazyme-Regulated Knockdown (SHARK) system to perturb a putative ubiquitin-conjugating enzyme E2, identifieda s a top marker of G1 phase. When depleted, we observe a significant growth reduction and kinetoplast defects. Together, these findings establish the first phase-resolved single-cell transcriptomes for the epimastigote cell cycle and metacyclogenesis, paired with functional validation, providing a powerful lresource for understanding parasite biology.

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