1

ASSESSMENT OF UNDER-FIVE MALARIA CASE MANAGEMENT IN ZAMBIA: RESULTS FROM A NATIONWIDE HEALTH FACILITY SURVEY

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Since 2003, the National Malaria Control Center in Zambia has implemented artemether-lumefantrine (AL) as first-line treatment for uncomplicated malaria and has expanded the availability of malaria testing, both rapid diagnostic tests (RDTs) and microscopy, to all districts. Previous case management surveys in 2004 and 2006 revealed underuse of diagnostics and inappropriate use of drugs. To assess health worker performance of correct malaria diagnosis and rational use of antimalarials, a cross-sectional nationwide cluster sample survey of health facilities was conducted in 2011. The survey included facility audits, health worker interviews, observations of consultations, and patient exit interviews. Eligibility criteria included patients visiting the outpatient health facility for an initial consultation with a health worker who were willing to provide consent. A total of 148 (88%) of targeted 168 health facilities were included. 872 patients seeking care had suspected malaria (history of fever and/or temperature ≥37°C); 51% of patients with suspected malaria were <5 years old. Nearly 71% of health facilities had diagnostic capacity, either RDTs (69%) or microscopy (12%). AL availability on the survey day was high across all health facilities (87.4%). However, although at least one formulation of AL was available in 87% of health facilities, only 48% had all types of dose packs. Most children <5 y were assessed for presence of fever (98%), with 77% having their temperature taken. For children <5, only 46% were weighed and 33% were assessed for prior malaria treatment. 71% of children <5 with suspected malaria (n = 437) had a diagnostic test performed while 28% not suspected to have malaria (n = 80) were tested. Testing rates decreased from higher levels of the health system (hospital 78%) to lower (health post 60%). 86% of children <5 (n= 96) with a positive blood test received AL; 11% of children <5 (n=21) with a negative test received AL. There have been marked advances in malaria case management in Zambia, with respect to both diagnostic testing and availability of AL, though rational use of AL remains suboptimal in children <5. Interventions aimed at health facility, health worker and patient levels have potential to further improve appropriate use of diagnostics and first-line antimalarials.

2

SEASONAL CHANGES IN THE FREQUENCY OF FALSE-NEGATIVE RAPID DIAGNOSTIC TESTS BASED ON HISTIDINE-RICH PROTEIN 2 (HRP2)

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Rapid diagnostic tests (RDTs) for *Plasmodium falciparum* malaria provide an invaluable alternative to microscopy in areas where microscopy is not readily available and - as a result - now play a central role in most malaria control programs. However, the frequency of false-negative test results is controversial and is potentially confounded by both low parasite densities and seasonal changes. Our original studies of this question in 1996 suggested that 5% of smear positive individuals had false-negative RDT results, ≥50% of which were associated with spontaneous deletion of the sub-telomeric hrp2 gene. In contrast, studies performed in Dioro, Mali during 2012 suggest that >80% of smear-positive individuals had false-negative RDTs near the end of the dry season, which then rapidly decreased to 20% within 3-4 weeks after the start of the rainy season. The positivity of the RDT increases with the parasite density ($X2 = 176.48$, $p < 0.001$) during the end of the rainy season. In addition, studies performed recently in Senegal suggest that up to 12-15% of positive RDTs may be false-positives. Because RDTs play a central role in virtually all malaria control programs, we are now performing a collaborative prospective study to compare RDT and smear results in the same subjects at field sites in The Gambia, Senegal and Mali order to address this question.

3

ASSESSMENT OF DRIED *PLASMODIUM FALCIPARUM* SAMPLES FOR MALARIA RDT QUALITY CONTROL AND PROFICIENCY TESTING IN ETHIOPIA

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Millions of rapid diagnostic tests (RDTs) have been used for laboratory confirmation of suspected malaria to comply with the revised WHO recommendations for malaria endemic countries, but programs lack quality control (QC) processes to assess RDT performance under field conditions. We evaluated a novel dried tube specimen (DTS) method for preserving *Plasmodium falciparum* parasite samples at specific concentrations for use as QC samples for RDTs. In the laboratory, we showed DTS to be stable for > 12 weeks when stored at 4°C, 25°C or 35°C. When stored at 4°C, DTSs were stable for >18 months. To test the feasibility of storing and using DTS as QC and proficiency testing (PT) samples at the point of care, we set up a pilot study in the Oromia Region of Ethiopia. Replicate DTS samples containing 0, 500 and 1000 parasites/µl were prepared and stored at 4°C at a reference laboratory (RL) and at ambient temperatures at two other nearby health facilities (HF). At 0, 4, 8 and 12 weeks the DTS were tested on duplicate RDTs stored under manufacturer recommended temperatures at the RL and on RDTs stored under site-specific conditions at the two HFs. Reactivity of DTS stored at 4°C at the RL on RDTs stored at the RL was the gold standard for assessing DTS stability. A PT panel with one negative and three positive samples was administered at week 12. Performance of the panel was monitored with a checklist. At weeks 0, 8 and 12, DTS with malaria parasites stored at both the RL and HF were reactive on all RDTs tested in duplicate, and the DTS without malaria parasites were negative. However, at week 4, we identified RDTs stored at the RL that were non-reactive at 1000 p/µl although the same sample was reactive on HF-stored RDTs from the same lot; indicating possible faulty tests within the batch of RL-stored tests. All facilities passed the PT panel; however, addition of excess blood to the RDT was identified at one health facility. After four time points of testing spanning three months, we show that the DTS method has the potential to supplement other RDT QC methods.

4

PLASMODIUM FALCIPARUM HISTIDINE-RICH PROTEIN 2 IS A CEREBROSPINAL FLUID BIOMARKER FOR CEREBRAL MALARIA

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Presently cerebral malaria is diagnosed on the basis of *Plasmodium falciparum parasitemia*, altered mental status and retinal changes. The examination of the cerebrospinal fluid in cerebral malaria cases shows absent pleocytosis, normal protein and glucose. The immuno-PCR technique was adapted for malaria use by coupling a 65 base pair oligonucleotide to a monoclonal histidine-rich protein 2 (HRP2) antibody. A primary HRP2 antibody was coated onto wells, followed by blocking, then sample incubation. The conjugated HRP2 antibody-oligonucleotide was added and after incubation nonbinding antigen-antibody complexes were washed. The antibody-antigen complexes were released into hot water and then input into real-time PCR. The limit of detection was demonstrated to be 1 picogram per sample with specificity over 90%. We demonstrate in a group of Tanzanian children that HRP2 is present by a novel sensitive immuno-PCR HRP2 detection in 98% of (82/84) samples. A RDT using 10 microL of CSF was positive in 81% (68/84) of the samples. The geometric mean HRP2 concentration in CSF was 10 picograms / microL with an average of 20 picograms/microL and median of 10 picograms/microL. In a small set of 11 matched plasma and CSF samples the ratio of plasma to CSF HRP 2 was 168. The Plasma levels of HRP2 averaged 1550 picograms / microL. HRP2 is present in the CSF of patients with cerebral malaria in the range of 10 picograms / microL for which some RDT could be adapted to verify the diagnosis of cerebral malaria. Exclusion of HRP2 in the CSF of patients with severe malaria anemia and elevated peripheral HRP2 needs to be determined.

5

MULTIPLEX MULTI-ANTIGEN, MULTI-SPECIES, MICROSPHERE-BASED IMMUNOASSAY TO DETECT ANTIBODIES TO HUMAN *PLASMODIUM* SPECIES

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An ELISA test that simultaneously detects antibodies against several *Plasmodium* species while enabling species differentiation would be highly valuable for epidemiology and vaccine studies in areas of mixed infections and identification of malaria-exposed blood donors in non-endemic countries. Here we report a highly sensitive, multiplex ELISA based on recombinant proteins from *Plasmodium falciparum*, *P. vivax* and *P. malariae* for pan-species and species-differentiating detection of antibodies in individuals living in malaria-endemic regions in Ghana and Cambodia. The assay was developed using the Luminex xMAP technology, where eight recombinant antigens (*P. falciparum*: CSP, AMA-1, LSA-1 and $MSP1_{19}$; *P. vivax*: CSP, AMA-1 and $MSP1_{42;}$ and *P. malariae*: $MSP1_{19}$) were covalently coupled to carboxylated magnetic beads. The results were expressed as median-fluorescence intensity and cut-off titers were established using a pool of human plasma samples collected from 13 malaria-naïve US blood donors. Pooled plasma samples from 10 Ghanaians had high IgG titers to PfCSP, PfLSA-1, PfAMA-1 and $PfMSP\text{-}1_{19}$ in a dilution-dependent manner. There was a high concordance (Spearman's rank correlation, $\rho > 0.92$) between the multiplex and plate ELISAs. The assay accurately identified 50/50 blood film-positive samples from patients with malaria and 50/50 blood film-negative samples from asymptomatic individuals in Ghana; 96% had reactivity to at least four of the eight antigens tested. The assay also detected 100% of plasma samples from Cambodian patients with acute *P. vivax* (50/50) and *P. falciparum* (50/50) malaria. Furthermore, multiplexing of antigens had no demonstrable antigenic inhibition or cross-reactivity when compared to reactivity against the individual antigens. Implications of the results for cross-species reactivity, detection of mixed infections, and association of antigen-specific antibodies with current infection versus past exposure will be discussed.

6

FIELD VALIDATION OF AN AUTOMATED MALARIA PF/PV RDT READER AND DATA MANAGEMENT DEVICE IN COLOMBIA

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Misinterpretation of RDT results can hamper massive implementation of diagnostics in low resource countries, by decreasing diagnostic accuracy. Studies have shown more errors in interpretation when multiple assays are involved in the diagnostic strip, as in the case of malaria Pf/Pv RDTs. Lack of proper quality assurance is also perceived as a significant obstacle to the widespread implementation of RDT-based malaria management strategy, as recommended by WHO. As well, reporting of diagnostic events is very limited, imprecise and slow in most remote areas, impeding proper decision making by control program managers. Fio Corporation has developed a system to address both problems: improving quality of RDT based diagnosis by providing job aids for RDT processing, automated interpretation through digital technology and optimal real-time case reporting using over cell-phone transmission networks. A fully blinded study was conducted in malaria endemic areas of Colombia to test the diagnostic accuracy of the Deki Reader using SD Bioline malaria Pf/Pv RDT. Patient population consisted of males and females >1 y. o., with symptoms of acute malaria. Main statistical analysis by a third party included dx performance of RDT interpreted by device (DEV), dx performance of RDT interpreted by experts visual (VIS), and comparison of DEV and VIS. Reference standard: expert microscopy performed at a central location. RT-PCR was used as tie-breaker in discrepant results. A total of 1,770 patients were enrolled over a 3 month period. *Pv* infections predominated (65.2%). Percentage concordance between DEV and VIS was >98.5 and was similar for *Pf* and *Pv* interpretation. Data from devices reached the Fio Cloud in real-time and could be accessed by PI and study coordinator. The Sens and Spec obtained are similar to other publications. Fio System was shown to deliver an automated high diagnostic performance even for RDTs containing multiple assays in the same strip, as good as expert visual interpretation. The system was found to be user friendly, practical, reliable and accurate. DEV false positives represented <1% of results.

7

DEVELOPMENT OF A MOLECULAR BARCODE FOR *PLASMODIUM VIVAX*

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The ability to detect, monitor, and track individual parasite types is proving to be extraordinarily useful in *Plasmodium falciparum* malaria. Using our experience developing a molecular barcode tool for *P. falciparum*, we have developed a similar tool for the identification and tracking of *P. vivax* parasites. This tool will enable researchers to identify and follow individual parasite types in time and space, distinguish reinfection from recrudescence in drug trials, detect escapees in vaccine trials, estimate multiplicity of infection in patient samples and *in vivo* non-human primate infections, and estimate transmission intensity. The assay was developed using Real-Time PCR and High Resolution Melting (qPCR-HRM) technology, as in our experience this is a robust, inexpensive, field-deployable genotyping technology that allows both assay of individual single nucleotide polymorphisms (SNPs) but also detection of novel changes in the assay region. However, as the tool is comprised of SNPs, the assays can be created and run on any number of platforms, thus the validation of SNPs that are useful for global parasite analysis is independent of technology. We selected SNPs with a high minor allele frequency from available *P. vivax* genome sequence data to identify candidate SNPs for assay development. To develop the assays, neutral sites among 631 SNP candidates including those SNPs in intergenic, intragenic or in 4-fold degenerate coding sites were evaluated and yielded 395 candidate SNPs for assay development. A final set of 95 assays was developed as an initial test set with the goal of identification of ~24 assays that would have a high minor allele frequency (MAF) among globally diverse parasite populations. These assays have been validated against a number of test DNA samples (Brazil I, Mauritania I, North Korea, India VII) to ensure they detect both the wild-type and the alternate allele at a given nucleotide position. We then applied these assays to *P. vivax* containing patient samples from geographically distinct parasite populations from the Americas (Brazil), Africa (Ethiopia) and Asia (Sri Lanka) to empirically derive the MAF and to select a subset of these assays that differentiate individual parasites from among these populations.

8

RETINAL MICROVASCULAR CHARACTERISTICS IN DENGUE FEVER INFECTION

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Retinal vascular caliber and geometric changes were associated with vascular diseases. Acute dengue infection may trigger measurable retinal vasculature changes that correlate with dengue severity. We aim to compare retinal vascular parameters between dengue patients and healthy controls, and study the association between retinal micropathology and biomarkers of inflammation. 61 dengue patients were recruited and 127 age, gender, ethnicity and co-morbidity matched healthy controls were selected from the Singapore Prospective Study Program. Quantitative retinal vascular parameters (retinal vascular caliber, branching angle, tortuosity and fractal dimension) were measured using a semi-automated computer-based program SIVA (Singapore I Vessel Assessment, version 3.0, Singapore Eye Research Institute, Singapore) by trained technicians following a standardized protocol. Retinal vascular parameters were compared with complete blood counts, liver and renal function tests. Dengue patients were had larger retinal arterioles (154.88 vs. 145.09, $p<0.001$), higher total arteriolar fractal dimensions (1.27 vs. 1.24, $p<0.001$) and venular fractal dimensions (1.26 vs. 1.22, $p<0.001$), and more tortuous arterioles (7.43 vs. 5.39 [$x10^{-5}$], $p<0.001$) and venules (8.79 vs. 6.89 [$x10^{-5}$], $p<0.001$), compared with healthy controls.Among dengue patients, each 1 U/L increase in aspartate aminotransferase (AST) was independently associated with a 0.03 µm increase in retinal arteriolar caliber ($p=0.037$), a 0.05 µm reduction in retinal venular caliber ($p=0.046$) and a $6.55x10^{-8}$ increment in retinal arteriolar tortuosity ($p<0.001$). Borderline tests of significance were observed with alanine transferase , hemoglobin and albumin. There was no correlation with platelet counts. In conclusion, retinal imaging showed a specific micropathology in dengue patients. These retinal vascular parameters were associated with changes in AST.

9

METABOLOMICS-BASED DISCOVERY OF SMALL MOLECULE BIOMARKERS FOR NONINVASIVE DENGUE DISEASE PROGNOSIS

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Epidemic dengue fever (DF) and dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) have overwhelmed clinical care capacity for this disease worldwide. We are using liquid chromatography-tandem mass spectrometry (LC-MS/MS) to identify molecular features and characterize candidate small molecule biomarkers (SMBs) predictive of dengue disease outcome (DF or DHF/DSS) in acute-phase serum and noninvasive saliva and urine clinical specimens. Metabolomics analysis was conducted using a 6520 Agilent TOF LC (Hydrophilic interaction liquid chromatography-HILIC)-MS and data analysis softwares Masshunter and MassProfilerPro. Acute-phase clinical samples from Mexico (68 serum, 45 urine and 59 saliva samples) and Nicaragua (90 serum, 86 urine and 86 saliva samples) from DHF/DSS, DF, and non-dengue (ND) febrile disease have been analyzed. Multiple molecular features have been detected that differentiated DHF/DSS from DF ($p<0.05$ and fold change >4), DHF/DSS from ND, and DF from ND. For example, 25 molecular features have been detected so far that differentiate DHF/DSS from DF in Mexican patient acute phase serum specimens and 81 in Nicaraguan specimens. In urine, 22 and 37 molecular features and in saliva 56 and 11 molecular features have been identified that differentiate DHF/DSS from DF in Mexican and Nicaraguan specimens, respectively. Identities of certain molecular features have been predicted *in silico* by searches of online databases (HMDB and Metlin) using their accurate neutral masses. To confirm the identities of these compounds, standards have been purchased for comparison by LC-MS/MS. Thus far, five compounds have been identified by LC-MS/MS. Provocatively, some of these candidate SMBs are associated with endothelial permeability and barrier function, dengue virus replication in host cells, and other mechanisms that could condition severe disease outcome. Currently, an additional 360 Nicaraguan samples are being analyzed by reverse phase LC-MS and LC-MS/MS for presence of candidate SMBs predictive of severe dengue disease outcomes. Our ultimate goal is to identify metabolic biosignatures for DF and DHF/DSS in acute phase serum, saliva, and urine samples and to exploit this information to develop rapid, point-of-care diagnostics to identify patients at greatest risk for severe dengue disease for supportive care and early therapeutic intervention.

10

EFFECTIVE AND EARLY LABORATORY DIAGNOSIS OF DENGUE GLOBALLY

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Dengue is transmitted in 100 tropical countries, with close to 100 million people presenting with symptoms of mild or severe disease. The 2009 WHO guidelines establish that dengue diagnostic testing should be used to differentiate dengue from other acute febrile illnesses. For many years, IgM ELISA seroconversion and virus isolation were the gold standards for confirmation of a dengue case. However, most patients present with symptoms before IgM antibodies are detectable, requiring a second sample be obtained for conclusive results, and virus isolation takes at least 7 days for a confirmation. Because dengue patients usually present with symptoms when they are viremic, there have been significant efforts in developing and improving tests for detection of dengue virus during the acute phase of illness. The CDC DENV-1-4 Real Time RT-PCR Assay was approved by the FDA in May 2012 for diagnosis of dengue. The assay has been configured to determine the subtype of the infecting virus and for detection of all strains currently circulating worldwide. Our clinical study of 371 cases showed a positive percent agreement of 98.04% between the CDC DENV-1-4 RT-PCR assay and IgM seroconversion, and 97.92% between RT-PCR and E gene sequencing. Following the implementation of the CDC DENV-1-4 RT-PCR assay in 110 laboratories, we have assessed the reproducibility of the assay worldwide. We have also compared the sensitivity of this test with that of 10 other reference and commercial molecular assays to provide a better understanding of their sensitivity and usefulness for early diagnosis. These developments make it now feasible to diagnose dengue on a single sample during acute illness globally. To evaluate this new diagnosis paradigm we have assessed the sensitivity of RT-PCR in a clinical study of 1234 dengue specimens. Our results show that early diagnosis of dengue is feasible during the acute phase of the disease, without having to obtain two samples.

11

COMPETITION OF DENGUE VIRUS SEROTYPE 2 CLADES FROM NICARAGUA REVEALS A FITNESS ADVANTAGE IN *AEDES AEGYPTI* MOSQUITOES

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The four dengue virus (DENV) serotypes are transmitted by *Aedes aegypti* and *Ae. albopictus* mosquitoes, causing up to 96 million cases of dengue worldwide each year. We have previously reported on a clade replacement within the Asian-American genotype of DENV2, in which the NI-1 clade was replaced with clade NI-2B over three epidemic seasons in Managua, Nicaragua. Here, we studied the replicative ability of DENV2 NI-1 and NI-2B viruses in mosquito cell lines and F2-F4 *Ae. aegypti* mosquitoes reared from eggs collected in Managua. Several different pairs of NI-1 and NI-2B strains were assessed *in vitro* and *in vivo* via a competition assay to yield an indicator of replicative fitness. In these experiments, equal genomic copy numbers of clinical isolates of NI-I and NI-2B viruses were mixed and used to infect Aag2 *Ae. aegypti* cells or inoculated into *Ae. aegypti* mosquitoes via a blood meal. At different time-points post-infection (p.i.), cell supernatant or mosquitoes were harvested, viral RNA was extracted, and a 1-kb region in the NS1 gene containing single nucleotide polymorphisms (SNPs) that distinguish clade NI-1 from NI-2B was amplified by RT-PCR. Amplicons were sequenced, and the proportion of each strain was determined using PolySNP PERL script software, which measures the height of the peaks of the SNPs from a sequencing chromatogram. Co-infections of the NI-1 and NI-2B viruses in Aag2 cells consistently showed a significant replicative fitness advantage of NI-2B over NI-1. The mean proportion of NI-2B over NI-1 significantly increased from the input in the carcasses of infected mosquitoes at 3, 6, 7 and 14 days p.i.. Additionally, NI-2B disseminated more rapidly than NI-1 viruses into mosquito legs. Importantly, NI-2B viruses were also found in greater abundance in the salivary glands at 7 days p.i., and the trend remained through the last time-point collected on day 21 p.i.. Furthermore, the NI-2B strains were the dominant virus in a greater percentage of mosquito carcasses (75%, 55/73), legs (71%, 27/38), and salivary glands (76%, 23/30) on day 7 p.i. and other time-points from 3-21 days p.i.. Finally, NI-2B was dominant in the legs (80%, 12/15) and salivary glands (56%, 18/32) at 14 days p.i.. Together, these results demonstrate that NI-2B clinical isolates have a modest early fitness advantage over NI-I viruses in multiple tissues of the native vector, *Ae. aegypti,* which could have contributed to the clade replacement observed in Managua.

12

DENGUE VIRUS INFECTION OF DERMAL DENDRITIC CELLS INCREASES DURING ANTIBODY-DEPENDENT ENHANCEMENT AND IS MODULATED BY *AEDES AEGYPTI* MOSQUITO SALIVA

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The four dengue virus serotypes (DENV1-4) are transmitted via the bite of infected *Aedes aegypti* or *Ae. albopictus* mosquitoes, causing dengue, the most prevalent arboviral disease in humans. Primary DENV infection confers life-long protective immunity to the same serotype, while secondary infection with a distinct DENV serotype is a major risk factor for severe disease via serotype cross-reactive enhancing antibodies (antibody-dependent enhancement; ADE) or T cells. Skin dendritic cells (DCs) are sentinels of the immune system and, with monocytes and macrophages, are targets of DENV in humans; yet, little is known about initial DENV replication in the skin or subsequent viral spread. We established a novel intradermal (i.d.) infection model of C57BL/6 mice lacking the IFN-α/β receptor (*Ifnar*$^{-/-}$) with DENV2 strain D220 inoculated via the ear. Intracellular staining with monoclonal antibodies directed against DENV NS3 and envelope proteins revealed DENV replication in epidermal Langerhans cells and, for the first time, in dermal CD11b^{+} DCs and CD103^{+} DCs by flow cytometry. In a model of ADE, inoculation of sub-neutralizing DENV-specific monoclonal antibodies enhanced infection of dermal DCs 48 hours after i.d. DENV infection, increased morbidity, and induced mortality of *Ifnar*$^{-/-}$ mice. While probing for blood, mosquitoes eject saliva into the skin. Salivary gland extracts from female *Ae. aegypti* co-injected i.d. with DENV2 reduced morbidity of mice during primary, non-enhanced conditions, but exacerbated disease during ADE infection. We are investigating the impact of ADE and mosquito saliva on DENV infection in the skin, virus spread, the immune response, and disease severity. These studies will improve understanding of DENV transmission, systemic spread, and factors that influence disease outcome and may inform future vaccine development.

13

PROPHYLACTIC AND THERAPEUTIC HUMAN MONOCLONAL ANTIBODIES AGAINST DENGUE

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Dengue virus (DENV) is the leading cause of mosquito-borne viral disease in humans worldwide. Although primary infection with one of the four serotypes of DENV (DENV1-4) results in lifelong immunity, secondary infection with a different DENV serotype increases the risk for severe dengue, a potentially lethal disease. Antibody-dependent enhancement (ADE) theory posits that cross-reactive, sub-neutralizing levels of anti-DENV antibodies facilitate increased viral entry into Fcγ receptor-bearing cells, thereby increasing viral load and disease severity. The presence of multiple DENV serotypes, as well as the ADE phenomenon, has hindered vaccine and drug development. As a part of multi-center collaborative project, we have begun identifying and characterizing human monoclonal antibodies (hMAbs) to DENV that have led to identification of three classes of potentially therapeutic hMAbs consisting of 1) highly-neutralizing, serotype-specific hMAbs that bind a novel hinge epitope on the envelope (E) protein; 2) strongly-neutralizing, serotype cross-reactive hMAbs that target E; and 3) serotype cross-reactive MAbs that suppress the activity of enhancing Abs. We have previously reported that "enhancement-suppressing" MAbs engineered to be unable to bind the Fcγ receptor are therapeutically effective in an ADE mouse model of lethal disease when they are highly neutralizing AND displace pre-existing enhancing Abs. Here, we focus on the prophylactic and therapeutic efficacy of type-specific and cross-reactive highly neutralizing hMAbs in our dengue AG129 mouse model (interferon-α/β and -γ receptor-deficient mice). Mice administered a type-specific hMAb to DENV1 (hMAb 1F4) or DENV2 (hMAb 2D22) 24 hours prior to a sublethal DENV1 or DENV2 infection, respectively, showed robust reduction of viral load in serum and various organs compared to mice receiving the isotype control. Mice receiving the cross-reactive hMAbs 1N5 or 1C19 showed reduction in both DENV1 and DENV2 viral load. Evaluation of prophylactic and therapeutic efficacy against lethal challenge and against other DENV serotypes is underway. Further study of these and other hMAbs should identify the simplest mixture of hMAbs that can be used as a single product for prophylactically and/or therapeutically preventing disease caused by the four DENV serotypes and may identify epitopes useful for future structure-based rational vaccine design.

14

LOVASTATIN FOR ADULT PATIENTS WITH DENGUE

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Dengue is globally the most important arboviral infection of humans. Despite the burden it places on health systems throughout the tropical world there is no vaccine and treatment is limited to supportive care. There is increasing evidence that statins may have a useful adjunctive role in conditions like sepsis through stabilising effects on the endothelium. In addition there is evidence that they may have antiviral effects against dengue. We are conducting a randomised, double-blind, placebo-controlled trial of lovastatin in adult patients with dengue (ISRCTN03147572). The main aim of this study is to assess the safety of statin therapy but also aims to explore the effect of statin therapy against a variety of clinical and virological endpoints. The trial is being conducted in two phases with a planned dose escalation provided a safety review is satisfactory. As there are no data on which to base a sample size calculation, a target sample size of 300 patients was chosen based on clinical judgement and feasibility considerations. 29 participants completed the 5-day oral course of treatment in phase 1 (40mg/day). 1 participant withdrew from the study. There were 5 serious adverse events. These were 1 participant with diarrhoea, 1 with a urinary tract infection, 1 with hepatitis and 2 with mucosal bleeding. All these events resolved and none were thought to be directly related to the study drug. In view of favourable safety results, the DSMB recommended that we commence phase 2 of the study. Phase 2 (80mg/day for 5 days) of the study is currently underway. A dengue therapeutic would be a major advance in global health. In view of their favourable effects on the endothelium and their good safety profile statins are an attractive therapeutic candidate for patients with dengue.

15

GENETIC MARKERS FOR MONITORING FOR IVERMECTIN SUB-OPTIMAL RESPONSES IN *ONCHOCERCA VOLVULUS*

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Ivermectin (IVM) is commonly administrated to control filarial parasites. In recent years there has been evidence of sub-optimal responses to ivermectin, suggestive of possible developing resistance in *Onchocerca volvulus*. The phenotype of this sub-optimal responsiveness (SOR) has been an apparent loss of the reproductive suppressive effect of ivermectin, leading to rapid repopulation of skin with microfilariae (mf). If this change in responsiveness has a genetic base, it could lead to higher than expected levels of parasite transmission and could become a serious problem for Onchocerciasis control and elimination in some regions of Africa. Recently, macrocyclic lactone resistance had been confirmed in *Dirofilaria immitis* a close related filarial nematode of *O. volvulus*. The goal of our project was to find markers that could reliably predict the SOR phenotype in *O. volvulus*. As a first step, we used a whole genome approach on phenotypically well characterized pooled samples from Cameroon and Ghana to address this issue. A number of *loci* showed highly significant differences between good responder and low responder samples. From these *loci*, a blinded Sequenom analysis was conducted on 160 *loci* from 597 individual adult parasites which were well characterized with regard to the phenotypic responses to treatment (embryograms and host mf

repopulation rates) to assess their genotypes. Fisher exact test analysis was performed to find *loci* that had significant differences between the good and the low responder samples with the objective to identify *loci* that could be used as markers for SOR and suspected IVM resistance in *O. volvulus*.

16

PHENOTYPIC ADAPTATION TO DOXYCYCLINE EXPOSURE IN *WOLBACHIA* REVEALED BY RNA-SEQ AND LABEL-FREE QUANTITATIVE PROTEOMICS

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The efficacy of tetracycline antibiotics against the *Wolbachia* endosymbiont of filarial nematodes has been unequivocally demonstrated in both *in vitro* and *in vivo* studies. However, several weeks of treatment are required to deplete *Wolbachia* to a level that ultimately leads to permanent sterilisation or killing of the adult worms. The reasons for this are unclear, but they have contributed to reluctance by public health policy makers to integrate doxycycline into filarial control programmes. Previous studies have shown that drug penetration into filarial worms appears to be highly efficient. Hence, *Wolbachia* may have a phenotypic ability to adapt to tetracycline exposure in the absence of classical drug resistance mechanisms. In this study, we subjected an insect *Wolbachia* strain in an *Aedes albopictus* cell line to three days of doxycycline exposure, and RNA and protein were extracted from purified bacteria. The RNA was analysed by RNA-seq on the Illumina HiSeq platform, whereas proteins were quantified by label-free ion intensity scoring on an Orbitrap Velos mass spectrometer. Coverage of the *Wolbachia* genome was >95% by RNA-seq and >40% by proteomics (>30% quantifiable with ≥2 peptides). The transcriptome exhibited massive suppression after treatment, with the downregulation of almost 400 transcripts, although a small number (50) were significantly upregulated. The most profoundly downregulated transcripts encoded heat-shock chaperones, tRNAs, the rod-shape determining protein RodA, and a large number of hypothetical proteins. Conversely, upregulated transcripts were dominated by those encoding ribosomal proteins, protein translocases and global regulators such as cold-shock protein and SurE. At the proteomic level, a small number (~30) of proteins were significantly downregulated in categories dominated by DNA repair, phospholipid synthesis and iron-sulphur cluster assembly, whereas no proteins were upregulated. Thus, although *Wolbachia* can extensively regulate its transcriptome in response to doxycycline exposure, the limited changes to the proteome could be determined by (a) intrinsic protein stability following inhibition of translation, and/or (b) controlled degradation of proteins by *Wolbachia* to limit cellular damage incurred by oxidised membrane lipids and the labile iron pool.

17

RAPID *WUCHERERIA*-SPECIFIC WB123-BASED IGG4 IMMUNOASSAYS AS SURVEILLANCE TOOLS FOLLOWING MASS DRUG ADMINISTRATION

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The Global Program to Eliminate Lymphatic Filariasis has an immediate need for rapid assays to detect ongoing transmission of LF following multiple rounds of mass drug administration (MDA). Current WHO guidelines support the use of the ICT antigen card test that detects active filarial infection but does not detect early exposure. Recent studies found that antibody based assays better serve this function. In the current study two tests, a rapid IgG4 ELISA and a lateral flow strip format, were developed based on the highly sensitive and specific *Wuchereria bancrofti* (Wb) antigen Wb123. Comparison of Wb-infected (n=95) to uninfected patients (with or without other helminth infections, n=289 for ELISA and n=279 for strips) determined both tests had high overall sensitivity (ELISA: 93%; strips: 92%) and specificity (ELISA: 97%; Strips: 96%). When the Wb-infected group was compared by ELISA to those with other filarial/helminth infections, the specificity was 92% with *Onchocerca volvulus* as the comparator, and 100% for both *Loa loa* and *Strongyloides stercoralis*. Comparison to parasite-uninfected individuals (both from helminth-endemic and -nonendemic countries) also showed 100% specificity. In addition, the geometric mean response by ELISA of Wb-infected patients was significantly higher than those without Wb infection ($p < 0.0001$). The specificity of the lateral flow strips was very similar and showed great stability between 20 minutes and dry reads assessed after 24 hours. Comparison of the 2 assays showed strong consistency with discordant results in only 3.5% of the 374 sera tested. Furthermore, both the Wb123 ELISA and the lateral flow strips had high positive and negative predictive values, giving valuable information on the needed survey size of a population to be relatively certain whether or not transmission is ongoing. These highly sensitive and specific Wb123-based IgG4 immunoassays should be useful tools for post MDA monitoring.

18

HIGH PRESSURE FREEZING/FREEZE SUBSTITUTION FIXATION IMPROVES THE ULTRASTRUCTURAL ASSESSMENT OF *WOLBACHIA - BRUGIA MALAYI* INTERACTION

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Wolbachia endosymbionts are essential for growth, reproduction and survival of many filarial nematode parasites. These α-proteobacteria were discovered in filarial parasites by transmission electron microscopy in the 1970's using chemical fixation methods. We postulated that improved fixation methods might reveal new information regarding *Wolbachia* motility and interactions between nematode cells and the endosymbiont. High pressure freezing/freeze substitution (HPF/FS) significantly improved fixation of *Brugia malayi* and *Wolbachia* resulting in much better visualization of membrane structures and different morphological forms of *Wolbachia*. Pleomorphy was observed for *Wolbachia* with regard to size, shape, and electron density of the cytoplasm. We also observed variability in the appearance of the nucleoid, vesicle formation, the number of membranes surrounding the bacteria, the space between membranes, and in patterns of *Wolbachia* aggregation. Endobacterial cytoplasm is surrounded by concentric, bilayer membranes. Vesicles with identical membrane structures were identified adjacent to the endobacteria, and multiple bacteria were sometimes enclosed within a single outer membrane. Immunoelectron microscopy showed that *Wolbachia* surface protein-1 was present in all of the membranes that enclose *Wolbachia* and structures that we consider to be *Wolbachia*-derived vesicles. *Wolbachia*-associated actin tails were not observed. *Wolbachia* motility may be explained by their residence within vacuoles, as they may co-opt the host cell's secretory pathway to move within and between cells. Seven days of tetracycline administered to experimentally infected gerbils reduced the number and size of *Wolbachia* clusters in L4 parasites and reduced the number of *Wolbachia* associated vesicles. Surprisingly, more glycogen was seen in the lateral chords of treated worms than those of untreated control worms. HPF/FS significantly improved the preservation of filarial tissues for electron microscopy to reveal membranes and subcellular structures that may be crucial for exchange of materials between *Wolbachia* and cells in the nematode host.

19

A PROTEOMIC ANALYSIS OF THE BODY WALL, DIGESTIVE TRACT, AND REPRODUCTIVE TRACT OF *BRUGIA MALAYI*

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Filarial worms are parasitic nematodes that cause devastating diseases such as lymphatic filariasis (LF) and onchcerciasis. Like all nematodes, filariae are pseudocoelomates with complex anatomy. To better understand the basic biology of filariae and to provide insights for drug and vaccine design, we conducted a proteomic analysis of different anatomic fractions of *Brugia malayi,* a causative agent of LF. Approximately 500 adult female *B. malayi* worms were dissected through a dissecting microscope and fine tipped forceps into three fractions: body wall, digestive tract, and uterine tract. Hematoxylin and eosin staining confirmed validity of the dissection technique. Samples were then homogenized and proteins extracted, desalted, trypsinized, and analyzed by microcapillary reverse-phase liquid chromatography-tandem-mass spectrometry. In total, we identified 4,785 *B. malayi* proteins. While 1,894 were identified in all three anatomic fractions, 396 were found only within the digestive tract, 114 only within the body wall, and 1,011 only within the uterine tubes. Gene set enrichment analysis revealed that the body wall is enriched in structural proteins, neuromuscular proteins, and proteins involved in energy metabolism. Proteins enriched in the intestinal tract included many metabolic enzymes and transporters, consistent with the concept that the gut of *B. malayi* is heavily involved in digestion and absorption. As expected, proteins enriched in the uterine tubes were primarily sheath proteins and proteins involved in the cell cycle. In assessing the intestinal tract for possible vaccine targets, we identified 3 proteolytic enzymes and 5 transporters that are likely expressed on the surface of intestinal epithelial cells and not in the other two anatomic fractions. Because these intestinal proteins may be physiologically important for digestion and absorption, and because they are not present on the body wall, they may represent "cryptic" antigens that are not typically encountered by the immune system yet may be effective if used as vaccine candidates.

20

MOUSE MODELS OF FILARIAL LYMPHODEMA REVEAL A THERAPEUTIC ROLE FOR VEGFR3 LIGATION

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The filarial pathologies, hydrocoele and elephantiasis, affect 40 million people ranking filariasis as the major global cause of secondary lymphoedema (LE). There are limited treatment options for secondary LE. We have developed two murine pre-clinical filarial LE models to test interventions that may improve lymph insufficiency mediated by filarial infection: an acute filarial dermal inflammation model within C57BL.6-Prox1GFP mice and a chronic *Brugia malayi* infection model in BALB/c SCID mice. In the former model, *B. malayi* adult female worm extract (BmFE) is introduced as an inflammatory stimulus in ear dermis. GFP expression targeted to lymphatic endothelium enables imaging of lymphatics during inflammation. In both models, optical imaging systems have been adopted to record changes in vascular leakage and lymph flow following inflammation or infection. Introduction of BmFE induced ear thickening and CD11b+ inflammatory infiltrates into dermal tissue accompanied by enhanced pro-fibrotic TGFβ, IL-13 and pro-angiogenic Vascular Endothelial Growth Factor (VEGF)A, C and D protein levels. The impact of filarial inflammation was a significant increase in CD31+ blood vascularity / Prox1+ lymphatic vascularity, increased blood permeability, increased collagen deposition and retarded lymph flow. Administration of rVEGFA164 co-incident with BmFE worsened some aspects of filarial pathology, including collagen deposition, whereas specific VEGF receptor 3 (VEGFR3) agonist, rVEGFC156S, reduced ear swelling, restored the balance of blood / lymphatic vascularity, reduced vascular leakage and collagen deposition. In the *B. malayi* SCID model, impaired lymph flow from the superficial lymphatics to the draining lymph nodes (dLN), dermal backflow of lymph and tortuous collateral lymphatics were recorded in ~50% of mice from +12 weeks following experimental *B. malayi* infections but not in immune competent WT mice. Treatment of *B. malayi* infected SCID mice exhibiting lymphatic pathology with rVEGFC156S for +7 weeks significantly improved lymph flow to the dLN by week 28. In conclusion, two models of filarial pathology have been developed that are amenable to longitudinal testing of lymphatic function following therapeutic interventions. Proof of principle data demonstrates that targeting the pro-lymphangiogenic VEGFR3 pathway may prove beneficial in restoring lymphatic function in filarial and non-filarial LE.

21

ANTIBIOTIC CHEMOTHERAPY OF ONCHOCERCIASIS: IN A BOVINE MODEL, DEPLETION OF THE *WOLBACHIA* ENDOSYMBIONT TRIGGERS PROFOUND DOWNREGULATION OF NEUTROPHIL ANTIMICROBIAL PROTEINS

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Onchocerciasis is currently controlled by annual mass drug administration of a single anthelminthic, ivermectin. Although this drug is highly effective at reducing disease symptoms, it does not kill the long-lived adult filarial worms (*Onchocerca volvulus*), necessitating repeated treatments for >15 years. Both *O. volvulus* and the closely related bovine parasite *Onchocerca ochengi* have a mutualistic relationship with the intracellular bacterium, *Wolbachia*. Clearance of this symbiont using tetracycline leads to killing of adult *Onchocerca spp.* approximately one year post-treatment. However, the precise mechanism of action remains unclear. In this study, we treated *O. ochengi*-infected cattle with a short, ineffective oxytetracycline regimen or prolonged, adulticidal therapy. Female worms were removed from nodules in bovine skin at three time-points (0, 12 and 36 weeks post-treatment), and protein extracts were subjected to label-free, quantitative proteomics on an Orbitrap Velos mass spectrometer. Approximately 1,500 proteins were quantifiable per sample, with 30% derived from the worm, 70% from the bovine host, and <1% from *Wolbachia*. Around 100 proteins were differentially regulated, of which the vast majority were host-derived, although *Wolbachia* protein levels were significantly reduced in the prolonged treatment group as expected. Conversely, there was little impact on the expression of worm proteins, except for a glutathione S-transferase and small number of intracellular signalling proteins. The largest group of downregulated bovine proteins were neutrophil-derived antimicrobial proteins, particularly cathelicidins, azurocidin and cathepsin G. However, other processes were also regulated in the mammalian host, including apoptosis, iron metabolism and the production of S100 inflammatory proteins. These data support the hypothesis that *Wolbachia* induces an ineffective neutrophilic response that is disrupted by antibiotic therapy, ultimately leading to immune clearance of the worms and nodule resolution.

22

GOLD FOR OLD PROTOZOANS: DRUG DISCOVERY FOR PARASITIC DIARRHEAL DISEASES

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Entamoeba histolytica, Giardia lamblia, and *Cryptosporidium* cause amebiasis, giardiasis, and cryptosporidiosis, three of the most common diarrheal diseases worldwide. The majority of patients with amebiasis and giardiasis are treated with a single class of drugs, the 5-nitroimidazoles, particularly metronidazole. Metronidazole has several adverse effects, and drug resistance is a growing concern in multiple protozoa. Nitazoxanide, the only FDA-approved drug for the treatment of cryptosporidiosis, is effective in the treatment of immunocompetent patients and partially effective for immunosuppressed patients. Therefore, finding additional drug targets is important for such significant causes of morbidity and mortality. To accelerate the identification of amebicidal and giardicidal compounds, we developed, optimized, and employed a high throughput screening methodology to survey large diverse compound libraries for their cytotoxicity to *E. histolytica* and *G. lamblia*. Our research identified an FDA-approved oral, inexpensive, gold-containing drug, auranofin, effective against *Entamoeba, Giardia*, *Cryptosporidium*. Our studies confirmed that auranofin targets *Entamoeba* and *Giardia* thioredoxin reductase, an enzyme involved in reactive oxygen species detoxification. This is a new mechanism of action for this drug in the treatment of amebiasis and giardiasis. Additionally, auranofin inhibited growth and survival of metronidazole-resistant *G. lamblia* isolates. Auranofin was found efficacious against *Cryptosporidium parvum* with EC50 about 2 μM, which was comparable to nitazoxanide, the current drug of choice. In vivo efficacy of auranofin at a low oral dose in a hamster amebic liver abscess model and mouse cecal amebic *coli*tis model documented decreased liver damage, reduced number of parasites and inhibition of detrimental host inflammatory response. Auranofin was also efficacious *in vivo* against infection with *G. lamblia* isolates in suckling mice and adult gerbil model. Based on these findings, auranofin has now received an Orphan Drug status from the USFDA for the treatment of amebiasis. Since the drug has been in clinical use for more than 25 years, the cost and development time for this "repurposed drug" can be significantly reduced. A clinical trial of auranofin for human amebiasis and giardiasis is currently under consideration.

23

STRUCTURE-AIDED EXPLORATION OF 5-AMINOPYRAZOLE-4-CARBOXAMIDE FOR SELECTIVE THERAPY OF *APICOMPLEXA*N INFECTIOUS DISEASES

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Effective treatment of infectious apicomplexan diseases is a formidable public heath challenge that will require new therapeutic strategies. Apicoplasts' Calcium dependent protein kinases (CDPK) are especially promising because orthologs are absent in mammalian genomes. Unlike most mammalian kinases, many apicoplast CDPKs have small gatekeeper residues within their ATP binding site, which render them more sensitive to the pyrazolopyrimidine scaffold of the bumped kinase inhibitor (BKIs). We have expressed and purified Apicoplast CDPK homologues from Babesia bovis, *Cryptosporidium parvum*, *Eimeria teneca, Neospora caninium*, *Plasmodium falciparum* and *Toxoplasma gondii*. In our continued efforts to explore alternative scaffolds for designing selective agents with favorable physicochemical and pharmacological profiles for drug discovery, we report here, the design of Apicoplast CDPK inhibitors based on the 5-aminopyrazole-4-carboxamide core using structure-based approach that specifically exploit apicoplasts' atypical CDPK gatekeeper pocket. A select group of compounds with low nanomolar IC_{50}s against parasite CDPK1s were further evaluated based on their inhibition of a mammalian kinase with small gatekeeper residue (Src), inhibition of *T. gondii* cell proliferation, and cytotoxicity against a mammalian cell line (CRL8155). Some of the compounds exhibit a few thousand folds of selectivity over Src with sub-micromolar activities against parasite proliferation, yet all of them seem to have low toxicity to mammalian cells. These compounds are good candidates for further investigation on pharmacological properties and efficacies in animal models.

24

RECOMBINANT EXPRESSION, SUB-CELLULAR LOCALIZATION AND BIOLOGICAL CHARACTERIZATION OF *BABESIA MICROTI* APICAL MEMBRANE ANTIGEN 1

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The incidence of *Babesia microti*, the primary causative agent of human babesiosis, is increasing in the United States. In apicomplexan parasites, apical membrane antigen 1 (AMA1) is a type I transmembrane protein and a component of a multiprotein complex that forms the moving junction involved in RBC invasion and that performs various other functions. By using the RACE system, we have isolated the full B. *microti* (BmAMA1) gene and determined its nucleotide and deduced amino acid sequence. It contains an N-terminal signal sequence, an ectodomain, a transmembrane region and a short cytoplasmic domain. BmAMA1 revealed a 35%, 31% and 32% similarity with *Plasmodium falciparum, P.vivax,* and *T. gondii* respectively. The twelve cysteine residues in BmAMA1 are conserved and aligned with *P. falciparum*, *P. vivax, B. bovis,* and *B. divergens.* Importantly, no polymorphism was detected in *Bm*AMA1 gene sequences obtained from seven human parasite isolates from *B. microti* infected individuals. The recombinant ectodomain expressed in *E. coli* reacted in ELISA and Western Blot with sera from *B. microti* infected individuals. In addition, immunization of mice with a DNA plasmid encoding for partial ecotodomian generated high levels of ELISA and IFA IgG anti-BmAMA1 antibodies. This antibody was used to demonstrate the surface localization of the AMA1 on *B. microti* parasites by immuno-fluorescence analysis and immuno-electronmicroscopy. *In vitro* binding studies indicated that BmAMA1 binds to trypsin and chymotrypsin sensitive receptors on human RBC membranes. Mouse anti-BmAMA1 antibodies exhibited 78% invasion inhibitory activity *in vitro* performed with free *B. microti* merozoites and human RBC in a 24 hour assay. Thus, given the possible role of BmAMA1in invasion of *B. microti* parasites in RBC, its ability to induce growth inhibitory antibodies, and presence of antibodies in naturally exposed individuals, this molecule appears to be an attractive vaccine candidate.

25

IDENTIFICATION OF TRANSCRIPTIONAL CIS-REGULATORY MOTIFS IN *THEILERIA PARVA*, AN INTRACELLULAR *APICOMPLEXA*N PARASITE OF CATTLE IN SUB-SAHARAN AFRICA

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Molecular regulation of stage differentiation in apicomplexan parasites has shown promise as a new approach for disease control. However, very little is known about transcriptional regulation in these pathogens, which are remarkable in their lack of canonical transcription factors and regulatory motifs. *Theileria* species are distinctive among apicomplexans in their apparent lack of enrichment for the binding site of AP2 transcription factors, believed to be the principal transcription factors in *Apicomplexa*. Therefore, *T. parva* is ideal as a model system for the discovery of novel apicomplexan-specific motifs involved in transcriptional control. The ability to identify the precise genomic coordinates of the start and end of transcripts, facilitated by RNA-seq technology, has the potential to improve our ability to identify transcription cis-regulatory motifs. *T. parva* infections result in the death of >1million cattle per year, with substantial economic impact on subsistence farmers in sub-Saharan Africa. The parasite's life cycle is complex. A sporozoite stage is transmitted to the mammalian host by a tick vector, followed by an intra-lymphocytic schizont stage and an intra-erythrocytic, infectious piroplasm stage. The schizont stage induces a leukemia-like proliferative and metastatic phenotype, the primary cause of pathogenesis. The transcriptional control of stage differentiation is critical for parasite transmission, colonization and pathogenesis. Here, we describe a method for identifying cis regulators of transcription in the *T. parva* genome, by searching for motifs enriched in regions surrounding the transcription start and termination sites as revealed by RNA-seq data. Our approach has resulted in the identification of a novel motif for which the location relative transcription termination site is conserved. We also found two previously known motifs, further validating for our approach. Ongoing experiments will determine the applicability of this approach to the identification of regulatory motifs in other apicomplexa, such as *Babesia* and *Cryptosporidium*.

26

CONGENITAL TOXOPLASMOSIS IN BRAZIL: MODELING THE COST OF MATERNAL SCREENING

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Toxoplasma gondii is a protozoal parasite infecting a high proportion of the world's population, although infection is generally asymptomatic in immunocompetent people. Congenital infection can result in fetal death or mild to profound visual, cognitive, and hearing impairment. A decision-analytic model applying the European protocol of universal maternal screening/ treatment to the low-prevalence US population found cost saving of $1 billion and prevention of avoidable injury in thousands of children every year. Using TreeAge Pro Suite software, we constructed a decision-analytic model to estimate costs of untreated toxoplasmosis and costs of screening, treatment, and follow-up for 3 high-prevalence Brazilian states. The model includes probabilities of maternal and fetal infection, fetal loss due to congenital toxoplasmosis (CT), post-natal infection, distribution of visual, hearing, and central nervous system injury, treatment efficacy, and non-probabilistic variables, such as costs of screening tests and treatment. Brazil has very high prevalence of toxoplasmosis, from 30% to 80% in different states, with different ecologies and quality of water and sanitary infrastructure. High adult prevalence is associated with high incidence during pregnancy due to acquisition in adolescence and young adulthood. High incidence of CT is compounded by a more virulent strain than found in Europe. The Brazilian strain affects 1 in 500 births and also can produce blindness when acquired post-natally, even in immunocompetent persons. Clinical experience in Brazil indicates that the local strain, if untreated, produces more profound injuries than the European strain, but that prenatal treatment is equally effective in preventing or mitigating injury. High levels of exposure, including from the water supply, make pre-natal and post-natal incidence a serious public health problem. In this high-incidence population, maternal screening is found to be cost-saving. Universal screening also has spillover benefits in community education, reducing post-natal infection and visual injury.

27

NOVEL CALCIUM DEPENDENT PROTEIN KINASE INHIBITORS AS A LEAD COMPOUNDS FOR TREATING CRYPTOSPORIDIOSIS

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The protozoan *Cryptosporidium* infects intestinal cells causing cryptosporidiosis, despite its high morbidity current therapies for this illness have limited efficacy. Thus new drugs are needed. Most of the current studies for drug development in cryptosporidiosis are based in the selective inhibition of vital targets, unfortunately most of these results comes from In vitro studies and few studies *in vivo* are available, therefore is needed test the efficacy of novel drugs in animal models. Calcium dependent protein kinases (CDPKs) are essential enzymes in the biology of protozoan parasites. CDPK1 was cloned from the genome of *Cryptosporidium* and potent inhibitors have been developed. Based in the structural observations and biochemical we hypothesized that a pyrazolopyrimidine derivate-1294 recently synthesized would be useful to treat cryptosporidiosis. We evaluated the anti-cryptosporidial efficacy of 1294 in human cells as well as in a SCID mice model. We showed that compound 1294 significantly reduce parasite infection. In the animal model, it markedly reduced the number of animals infected and parasite burden and decreased epithelial damage. Subsequently, we have tested additional CDPK-1 inhibitors and found even more potent inhibitors with submicromolar inhibitory concentrations. Thus this class of inhibitors are important leads for the development of more potent treatments of cryptosporidiosis.

28

EMERGING *SARCOCYSTIS NESBITTI* INFECTION IN HUMAN

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Sarcocystis infection is a rare endemic disease of humans. In recent years, increasing number of travelers to South East Asia contracted an acute muscular *Sarcocystis*-like illness upon returning to their respective countries. Here, we report a potentially large outbreak of human *Sarcocystis nesbitti* infection amongst a group of college members who had visited an island of the west coast of Malaysia. An outbreak investigation was undertaken following the presentation of symptomatic persons to the University of Malaya Medical Center. Several of the patients developed facial temporalis myositis and others complained of specific muscle pain. Over 90% of the college members came down with relapsing fever, myalgia, headache, cough, nausea, vomiting and diarrhea within

three weeks upon returning. Muscle biopsies were obtained from several of these patients. The muscle tissues were ground with sterile glass beads using the Precellys 24 homogenizer. Molecular detection for possible microbial infections was performed on the tissues. Nucleic amplification of the tissue biopsies consistently gave *Sarcocystis* DNA fragments with sequences that matched that reported for *S. nesbitti*. Phylogenetic tree constructed using the sequences along with those available in the GenBank placed the sequences in a clade together with *S. atheridis*. Our findings highlight the emerging importance of *S. nesbitti* as a potential human pathogen.

29

ARGININE AVAILABILITY AND THE HOST IMMUNE RESPONSE TO *GIARDIA LAMBLIA* INFECTION

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Arginine plays an essential role in innate immunity as it is required for the production of nitric oxide (NO) by nitric oxide synthase (NOS). NO is toxic to many pathogens, and limiting NO production is a survival strategy employed by numerous microbes. *Giardia lamblia*, a protozoan parasite of humans and many other animals causes the diarrheal disease giardiasis. Previous studies indicate that NO inhibits parasite growth and that *Giardia* consumes arginine for the production of ATP. This consumption of arginine by *Giardia* could serve as a means by which it inhibits the host immune response. Another mechanism of arginine depletion employed by pathogens is the induction of host arginase (ARG). However host ARG activation has never been explored in *Giardia*. We hypothesize that *Giardia* infection induces ARG expression limiting the arginine available for NO production. In this study, real time PCR was used to measure changes in ARGI and NOS2 expression in mouse intestine following infection. Immunohistochemistry and flow cytometry were performed to identify ARGI and NOS2 expressing cells in the intestine. While infection leads to changes in expression of both ARGI and NOS2, ARGI precedes a significant increase in NOS2 expression. This increase in ARGI could indicate that *Giardia* directly stimulates the expression of host ARG. Flow cytometry confirmed the presence of both enzymes in macrophage cells of the intestinal lamina propria. To determine if *Giardia* can directly induce ARG expression in macrophages, we are conducting *in vitro* stimulations of RAW 264.7 macrophage-like cells with *Giardia* extract. Studying the role of arginine in *Giardia* infection will yield information on how arginine availability influences host and parasite biology, the role of nutrition in determining host susceptibility to disease, and novel treatments for giardiasis.

30

CLOUD-BASED GIS TOOL EASES GRAPHICAL REPRESENTATION FROM MULTIPLE DATA SOURCES TO SUPPORT PLANNING AND IMPLEMENTATION OF NEGLECTED TROPICAL DISEASE (NTD) CONTROL ACTIVITIES

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Information on the geographical distribution of disease is essential to identify priority areas for intervention, estimate intervention needs and track progress in control. Yet, despite the availability of data for many countries, policymakers and program managers are generally unable to access the information in a geographic format for planning purposes. To address this deficiency a readily available, user-friendly interactive mapping tool has been developed to help the planning and implementation of Neglected Tropical Disease (NTD) control activities. The 3 variables most useful to the end user were determined to be i) endemicity status, ii) treatment activity and iii) treatment coverage. A geo-database was designed to serve as the bridge between the data and the mapping tool. Then a mapping tool was built using esri java script to display from an individual's laptop computer an interactive map containing the agreed upon variables. Existing data sources from 4 separate programs representing the 5 'preventive chemotherapy' NTDs (lymphatic filariasis, onchocerciasis, schistosomiasis, soil transmitted helminths and trachoma) were linked to the geo-database so that endemicity status, treatment presence and treatment coverage could be readily visualized. The tool allows the user to selectively view multiple variables at once, identifying co-endemicity and intervention gaps. Linking data from multiple partners provides a robust understanding of the status of each disease. The sharing of this valuable information in a geographic platform provides a stepping stone for integrated work in country. The tool provides a mechanism for partners, regardless of GIS skills and software, to be able to generate useful programmatic maps. The design of this tool allows for data transparency among partners, facilitating data crosschecking among the multiple reporting mechanisms and providing valuable information for programmatic decision making.

31

EVALUATION OF FOUR YEARS IMPLEMENTATION OF THE SAFE STRATEGY (SURGERY, ANTIBIOTICS, FACIAL CLEANLINESS AND ENVIRONMENTAL IMPROVEMENT) FOR TRACHOMA CONTROL IN HANDENI DISTRICT, TANZANIA

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The World Health Organization recommends evaluation of the SAFE strategy after at least three years of implementation. We investigated prevalence of trachomatous inflammation-follicular (TF) and trachomatous inflammation-intense (TI) in children aged 1-9 years and prevalence of trachomatous trichiasis (TT) in people aged 15 years and above following four years of SAFE in Handeni district. Cluster random survey design was used to select the sample. Handeni district was stratified into four sub-districts. Ten villages (clusters) were randomly selected in each sub-district. Villages were stratified by hamlet and an equal number of households selected in every hamlet using systematic random sampling. In all household selected children aged 1-9 years were examined for TF and TI, and persons aged 15 years and above were examined for TT and corneal opacity (CO). Point prevalence estimates for TF, TI and TT were generated with adjustment for sampling probability and clustering. A total of 2,909 households were surveyed. The number of participants examined for trachoma signs were 5,301 children aged 1-9 years and 8,168 people aged 15 years and above. The overall prevalence of TF in Handeni was 2.6% (95% confidence interval [CI] 1.9-3.6) and varied by sub-district ranging from2.1% (95%CI 1.0-4.4) to 4.5% (95%CI 3.4-5.9). Overall prevalence of TI was 0.7% (95%CI 0.5-1.0) and ranged from 0.4% (95%CI 0.2-1.0) to 1.4% 95%CI 0.8-2.5) by sub-district. The prevalence of TT by sub-district ranged from 0.6% (95% CI 0.2-1.5) to 0.8% (95%CI 0.5-1.4), with the district prevalence of 0.7% (95%CI 0.5-1.1). Compared to the baseline prevalence (28.6%), there was a 90.9% decline of TF in Handeni district. The results of this survey suggest that trachoma is no longer a public health problem in Handeni district and therefore

Mass Drug Administration of Zithromax should be stopped according to WHO guideline. Continuation of F and E components of SAFE should be considered.

32

AN ECONOMIC EVALUATION OF INCREASING THE FREQUENCY OF IVERMECTIN TREATMENT FOR ONCHOCERCIASIS CONTROL IN AFRICA

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There has been a recent shift in onchocerciasis control policy in Africa, with intervention programmes changing their aim from disease prevention to elimination of infection. It has been suggested that switching to biannual (twice per year) ivermectin distribution might improve the chances of reaching elimination goals. However, the circumstances under which this strategy would be effective in African settings have not been assessed. We use a deterministic onchocerciasis transmission and disease model to explore the impact on human health, parasite populations, and programme cost of using a biannual treatment strategy in different epidemiological and programmatic scenarios in savannah areas, assuming that drug efficacy remains intact during the programme. We also explore the impact of switching to biannual treatment at different stages of ongoing annual treatment. Our projections indicate that although biannual (either from the start or by switching from annual) treatment would have only a small additional impact on health, it can notably reduce programme duration. The impact of biannual treatment is strongly related to pre-control endemicity, with greater projected benefits for higher initial endemicity. This particularly applies to highly hyperendemic areas, for which our projections indicate it would not be feasible to reach current operational elimination thresholds with annual treatment alone (due to residual transmission between annual treatments). We conclude that biannual ivermectin treatment may have a substantial benefit in terms of reaching elimination goals, potentially generating cost savings. However, projections regarding the benefit and cost of biannual treatment are highly sensitive to levels of systematic non-compliance, which have a larger bearing on the projected benefit of switching to biannual treatment than overall therapeutic coverage.

33

INCENTIVIZING COMMUNITY DRUG DISTRIBUTORS DURING MASS DRUG ADMINISTRATION CAMPAIGNS FOR NEGLECTED TROPICAL DISEASES: POLICY VS. PRACTICE

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Incentives - both monetary and non-monetary - are often provided to community drug distributors (CDDs) as part of Mass Drug Administration (MDA) campaigns under the national Neglected Tropical Disease (NTD) control programs. The rationale and costs behind the use of incentives vary between countries and programs. In certain countries the use of incentives is explicitly defined and incorporated into national policies on health service delivery, while other countries have developed more ad hoc approaches. The divergence between policy and practice and the disintegration of a standard approach within a country can lead to variation in incentive schemes and unintended financial and operational costs. To measure variation of CDD incentive schemes on costs and frequency of administration, a comprehensive document review and key informant interviews with program managers were conducted in 11 countries supported by the USAID-funded NTD Control program during 2006 -2012. Country specific definitions of incentives were recorded and monetary and non-monetary incentices were assessed for cost, purpose, recipient, and associated drug package. Results illustrate a complex interpretation of incentives used by National NTD control programs during 2006-2012. Country programs described three major purposes of CDD incentives: 1) compensate for time and effort, 2) motivate participation, and 3) improve performance. Incentive costs varied depending on the defined purpose of incentives, and the degree to which various categories of CDDs were involved in the distribution of drug packages. Per person incentive costs were greater among teachers, who were more likely to be targeted with incentives to compensate for time and effort than incentives provided to other CDD categories. No significant difference was observed between incentive costs and drug packages. This evaluation provides national NTD program managers with several case examples for understanding the potential impact of various CDD incentives on financial and operational costs when scaling-up MDAs to a national level.

34

VALIDATION OF TRACHOMA SEROLOGICAL TESTS IN HIGH AND LOW PREVALENCE VILLAGES OF KONGWA DISTRICT, TANZANIA

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WHO has set a goal for elimination of blinding trachoma as a public health problem by the year 2020 based on criteria defined by clinical examination for trachomatous inflammation- follicular (TF). Defining endpoints for trachoma programs can be a challenge since TF may persist in the absence of detectable bacteria. Antibody-based tests may provide an alternative testing strategy for surveillance during terminal phases of the program and for post-elimination surveillance. We have recently demonstrated high sensitivity and specificity of responses to two chlamydial antigens, pgp3 and CT694, in children living in a trachoma-endemic community of central Tanzania. To further understand how antibody tests could be used in a programmatic context, we compared antibody levels, clinical pathology (TF), and the presence of ocular *Chlamydia* DNA (using PCR) in areas with high, medium, and low TF prevalence prior to mass drug administration. All studies were conducted in Kongwa District, Tanzania. In the high TF prevalence setting, the overall TF prevalence among 208 children aged 1-6 was 43.2%, the PCR prevalence was 24%, and the seroprevalence was 63%. Fifty out of 52 (96%) PCR-positive and 80/97 (82%) TF/TI-positive individuals tested positive by serology. PCR-positivity remained relatively constant across all ages (range 20.5-28%). TF prevalence also did not increase with age (30.4% - 61%). In contrast, antibody responses showed a distinct age-dependent increase. By age 4, seroprevalence reached 80% and by age 6 the seroprevalance was 96% (32/33). We will compare these data to those found in medium (mean TF of 14.5%) and low prevalence (mean TF of 2.8%) villages of Kongwa District to further define how antibody responses can be used to monitor trachoma programs.

35

PILOTING SEROLOGICAL TOOLS FOR TRACHOMA POST-ELIMINATION SURVEILLANCE

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Trachoma, an ocular infection caused by *Chlamydia trachomatis*, is the leading infectious cause of blindness worldwide. Yearly mass drug administration (MDA) of azithromycin plays a central role in programs seeking to eliminate blinding trachoma as a public health problem by 2020. Decisions for starting and stopping programs are currently made based on the presence of trachomatous inflammation-follicular (TF) and trichiasis (eyelashes rubbing against the globe, TT). WHO guidelines do not currently include recommendations on post-MDA monitoring and surveillance, and currently there are no validated tools to carry out surveillance. We sought to test recently developed serological tools for trachoma in the sub-village of Kahe Mpya, Rombo District, Tanzania, where trachoma was declared eliminated in 2005. From 989 residents, 571 people were examined for clinical signs of trachoma. The overall prevalence of active trachoma (TF, trachomatous inflammation-intense [TI], or both) in the study population was 4.6%, with 21.5% exhibiting signs of scarring (TS), trichiasis, or corneal opacity (CO). The overall prevalence of TF and TI in participants <8 years (i.e. born after cessation of MDA) was 8.4% (peaking at 26.7% in 1-1.9 year olds). A higher proportion of these had TF (6.7%) than TI (1.7%). Dried blood spots and conjunctival swabs to identify the presence of bacterial were also collected as part of this study. Assessment of age-specific antibody responses to the previously identified antigens pgp3 and CT694 will provide critical information to determine if serological responses to chlamydial antigens may be an informative proxy for the presence of transmission in a post-MDA setting.

36

COMMUNITY PARTICIPATION IN WATER, SANITATION AND DEWORMING ACTIVITIES IN THE CONTROL OF BILHARZIA IN NYALENDA B, AN INFORMAL SETTLEMENT IN KISUMU CITY

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This study explored community participation in water, sanitation and deworming activities in the control of bilharzia in Nyalenda B, an informal settlement in Kisumu City where Bilharzia control was being implemented. Eight key informant interviews (KIIs) and focus group discussions (FGDs) were conducted. Each FGD was categorized by gender and age with participants from the nine sub-units in the study area. Participants included beach management workers, community health workers, church leaders, village heads, teachers, volunteers at various NGOs, fishermen, members from youth groups and support groups. The key informant interviews were conducted among Municipality health workers and front line health facility officers. Data was organized into themes and concepts in a narrative form and then analyzed using Atlas.ti. Most participants felt that project implementers did not involve them in key levels of project implementation (Information sharing, consultation, decision-making). This in turn led to unsustainable projects and unacceptance from the community. Participants also identified structures in the community that could be used as avenues of engaging the community in improving water and sanitation situation, for instance use of organized groups such as youth groups, gender based groups, adult women groups, farmers groups, merry-go rounds, and HIV support groups. Several factors were mentioned that hinder community participation including negative attitude from community members, poor monitoring and evaluation strategies which has lead to unsustainability of projects, limited disclosure of project details to community members, and over-dependence from the community. Poor drainage systems, low latrine coverage, broken pipes and leakage of the sewerage systems were the leading factors associated with poor water and sanitation conditions. Use of organizational groups and partnerships was cited as an important avenue of engaging community members towards improving water and sanitation activities. For effective community participation in water, sanitation and deworming activities, a multi-pronged paradigm is required that incorporates change of attitude, information sharing and consultation, improved monitoring and evaluation, transparency and accountability.

37

RECENT EXPOSURE TO MALARIA INDUCES CD4+ TH1 CELLS CO-PRODUCING IFNΓ AND IL-10

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Plasmodium falciparum infection is thought to induce potent immunoregulatory responses, but the precise mechanisms in humans are unclear. Potential mechanisms include the induction of traditional FoxP3$^+$ regulatory T cells and/or antigen-specific CD4$^+$ Th1 cells that produce IFNγ and IL-10, the latter of which have been found to limit excessive inflammation in other parasitic infections. To evaluate T cell populations in children living in a highly malaria-endemic area, peripheral blood mononuclear cells (PBMCs) were obtained from 78 HIV-uninfected 4-year old children with known malaria history from a longitudinal cohort study in Tororo, Uganda. The prior incidence of malaria in this cohort ranged from 0-11 episodes ppy, with a mean incidence of 5.4 episodes ppy. PBMCs were stimulated with media, PMA/ionomycin, *Plasmodium falciparum*-infected red blood cells (iRBC), or uninfected RBCs and assessed for surface marker expression, intracellular cytokine staining, and transcription factor expression and analyzed on an LSR2 cytometer (BD). After stimulation with iRBC, CD4$^+$ T cells producing IFNγ were detected in 86% of subjects. A majority of IFNγ-producing CD4$^+$ T cells simultaneously produced IL-10 (median 74%, IQR 62-80%) while a minority produced TNFα (median 28%, IQR 16-44%). IFNγ/IL10-producing CD4$^+$ T cells were associated with prior incidence (r=0.33, p=0.004) and inversely associated with days since last episode of malaria (r=-0.52, p<0.001). In multivariate analysis, time since last episode of malaria was the strongest predictor of the frequency of IFNγ/IL10-producing CD4$^+$ T cells (p=0.001). IFNγ/IL10-producing CD4$^+$ T cells expressed the Th1 master transcription factor T-bet, were of a transitional memory phenotype (CD45RA$^-$, CCR7$^-$, CD27$^+$), and did not typically produce TNFα or IL-2. Surprisingly, the prior incidence of malaria was inversely correlated with the frequency of CD4$^+$ CD25$^+$ FoxP3$^+$ T_{regs}, with more prior malaria associated with fewer T_{regs} (r= -0.33, p=0.006). In naturally exposed children, a higher frequency of IFNγ/IL10 co-producing CD4$^+$ T cells and a lower frequency of FoxP3$^+$ T_{regs} represent novel T cell correlates of exposure to malaria, and may play important modulatory roles in the development of antimalarial immunity.

38

MALARIA GENETIC *LOCI* MODULATING STRAIN-SPECIFIC INNATE IMMUNE RESPONSE AND VIRULENCE

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Clinical outcomes of a malarial infection are influenced by both host and parasite factors. The goal of the study is to identify malaria genetic *loci* modulating strain-specific innate immune response and virulence through analysis of cytokinomes and genome-wide gene mapping tools. Our analysis included the measurement of levels of plasma cytokines/chemokines (CC) and growth rate in mice infected with three *Plasmodium yoelii* strains having different virulent phenotypes and progeny from a genetic cross of lethal and nonlethal parents to investigate the effects of parasite factors on host innate immune response and pathogenesis. We showed that parasite's ability to induce CC was strain-dependent, inheritable, and critical for controlling *parasitemia*. Quantitative trait *loci* analysis and allelic replacement analysis also identified the multiple *loci* including the *P. yoelii* erythrocyte binding ligand as the major genetic determinants of the cytokine-mediated virulent phenotypes. These results provide important information for better understanding of malaria pathogenesis and for developing measures for disease control.

39

PLASMODIUM FALCIPARUM INDUCES CHANGES IN PLASMACYTOID DENDRITIC CELLS IN SYMPTOMATIC BUT NOT IN ASYMPTOMATIC PATIENTS DURING ACUTE INFECTION IN THE AMAZON REGION

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Dendritic cells (DCs) play an important role in the induction and regulation of immune responses via antigen-presentation, co-stimulation and production of cytokines and chemokines. In malaria, the functionality of DCs remains elusive because of immunomodulatory properties of the *Plasmodium falciparum* parasite. Changes in peripheral populations of DCs during acute *P. falciparum* malaria were recently characterized in Brazil and Thailand; but no data are available for *P. falciparum* infections in low transmission settings as the Peruvian Amazon. We characterized peripheral populations of DCs in uncomplicated malaria patients infected with *P. falciparum* and in healthy controls living in the same area of endemicity and exposed to relatively low levels of malaria transmission. Cryopreserved PBMCs from *P. falciparum* infected patients (symptomatic and asymptomatic) and endemic controls were stained with an antibody mixture containing lineage-specific mAbs to CD3, CD14, CD16, CD19, CD20, and CD56 conjugated with FITC (lin-FITC), antibodies to CD11c conjugated with APC and CD123 conjugated with PE, and antibodies to HLA-DR conjugated with PerCP. 50000 events were analyzed in a C6 Accury flow cytometer (BD). HLA-DR+ CD123+lin–cells were defined as plasmocytoid dendritic cells (PDC) and HLA-DR+CD11c+lin– as myeloid dendritic cells (MDC). The proportion of circulating MDC and PDC was reported. The results showed that the proportions of PDC were significantly reduced in *P. falciparum* symptomatic (8.02 %) and asymptomatic (11.19 %) patients than those controls (16.65 %). In asymptomatic patients there was no change in MDC (14.1 %) compared to controls (13.05 %) but in symptomatic patients there was a dramatic reduction in MDC (5.8 %). In conclusion, *falciparum* malaria induced a clear decrease in the proportion of circulating DCs with the plasmacytoid (CD123) phenotype in symptomatic and asymptomatic patients and myeloid (CD11) phenotype in symptomatic but not in asymptomatic patients compared to controls.

40

ANTIBODY-MEDIATED COMPLEMENT-DEPENDENT INHIBITION OF *PLASMODIUM FALCIPARUM* MEROZOITE INVASION IN HUMAN IMMUNITY TO MALARIA

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Antibodies play an important role in acquired immunity to malaria in humans, however the effector mechanisms that mediate their protective function are poorly understood. Although acquired and vaccine-induced antibodies can directly inhibit merozoite invasion and blood-stage replication of *P. falciparum*, many antibodies to merozoite antigens have little or no direct inhibitory activity, and antibodies that inhibit parasite growth are not strongly predictive of protective immunity to malaria. Other mechanisms of antibody-mediated immunity are likely to be important, but are not fully defined. Complement is an essential component of the adaptive humoral immune response for many pathogens, but its role in acquired humoral immunity to malaria is not known. We have investigated the potential role of complement in naturally-acquired and vaccine-induced immunity to malaria using novel approaches with antibodies from children and adults acquired through natural exposure and antibodies induced by human immunization with a major merozoite surface protein in a phase 1 clinical trail. We have established that human antibodies to merozoite surface antigens promote the deposition of complement to enhance the invasion-inhibitory activity of antibodies. We have defined the relationship between the levels and nature of IgG subclass antibodies and complement activity, and investigated the role of individual complement components required for inhibitory activity. Our results identify antibody-mediated complement-dependent inhibition of invasion as an important new mechanism in humoral immunity to malaria, and we have established a new assay for evaluating the function of acquired and vaccine induced human antibodies. We believe this marks a significant change in how we understand antibody-mediated protection from malaria, and has major implications for vaccine development and evaluation.

41

DEFINING CORRELATES OF PROTECTION FROM PLACENTAL MALARIA USING A PREDICTIVE MULTI-ASSAY APPROACH

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Malaria during pregnancy poses risk of serious health complications for approximately 85 million mothers and developing fetuses worldwide. *Plasmodium falciparum*-infected erythrocytes accumulate at the maternal-fetal interface of the placenta using the VAR2CSA adhesion molecule, creating a condition known as placental malaria. Antibodies against VAR2CSA improve pregnancy outcome and a vaccine based on VAR2CSA is feasible. However, VAR2CSA antibody function or functions that mediate protection from placental malaria are unknown. Knowing correlates of protection will expedite development and field-testing of a malaria vaccine for pregnant women. Therefore, the goal of the study was to identify correlates of protection from placental malaria. Plasma samples collected at delivery from Cameroonian women with (n=115) and without (n=345) placental malaria were screened in multiple assays to measure antibodies to recombinant full length VAR2CSA, its 6 DBL domains and 15 strain variants, the surface of infected erythrocytes, as well as IgG isotype, IgG avidity, FcγR-mediated opsonic phagocytosis, and antibody-mediated

inhibition of binding. Each assay was considered individually using receiver operating characteristic curve method to determine sensitivity and specificity. No correlation with protection was found using a single assay. We are in the process of analyzing all the assays in a multivariable logistic model, adjusting for important covariates and eliminating assays that do not significantly contribute to the overall model. Final model in a form of equation based on the selected assays will provide a way investigators can predict the probability a woman will have placental malaria at delivery. Results will define a correlate(s) of protection from placental malaria, which will provide a way to evaluate vaccine efficacy in field trials. In addition, clinicians will have a method for early identification of women at risk of placental malaria and therefore early intervention.

42

IMMUNE MEDIATED SELECTION OF *PLASMODIUM FALCIPARUM* IDENTICAL GENETIC VARIANTS VIA VARIANT SURFACE ANTIGENS

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As malaria transmission intensity has declined in some regions, *Plasmodium falciparum* parasite populations are displaying decreased genetic diversity. Additionally, the emergence of many parasites with identical genetic signatures has been observed both by molecular barcode genotyping and whole genome sequencing. We have monitored genetically identical parasite clusters from 2006-2012 in Thies, Senegal, and we have characterized these parasites to determine whether they are epigenetically and antigenically identical. This allows us to test the hypothesis that the emergence, decline, or expansion of these populations is mediated or modulated by the human host immune system. We focus on one cluster of identical parasites that was present in 24% of clinical isolates in 2008 and declined to 3.4% of clinical isolates in 2009. We studied the susceptibility of 2 representative common genetic signature (CGS) parasites to invasion inhibitory antibodies using Growth Inhibition Assays (GIA), and we have studied infected RBC IgG reactivity by variant surface antigen (VSA) flow cytometry. We find that the CGS parasites are similarly susceptible to invasion inhibition by patient IgGs from 2008 and 2009, arguing against invasion-blocking immunity being the selective pressure against these parasites in 2009. By VSA flow, these parasites are recognized to similar extents by plasma IgG from 2008 and 2009, but reactivity against both is dramatically increased in 2009. Such findings could imply that VSAs present on infected RBCs are the target of immune responses that, while permissive in 2008, selected against these parasites in 2009. As PfEMP-1 is a dominant component of the VSA response, we characterized the *var* genes expressed by CGS parasites by *var* Ups qRT-PCR and by sequencing using degenerate DBL domain primers. We observed that the CGS parasites expressed the same *var* Ups classes, marked by a striking upregulation of UpsA *var* genes and 2-cysteine containing PfEMP-1 molecules compared to non-CGS parasites. Taken together, our work indicates that there is selection against these common genetic variant parasites at the level of surface expression of VSAs.

42A

DOSE-RANGING EFFICACY RESULTS FROM THE TAFENOQUINE 'DETECTIVE' TRIAL: A RANDOMIZED, DOUBLE-BLIND, MULTI-CENTRE, PARALLEL-GROUP STUDY FOR THE RADICAL CURE OF SUBJECTS WITH *PLASMODIUM VIVAX* MALARIA

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Plasmodium vivax, which causes over 400 million malaria cases annually in malaria endemic regions of Asia, Central and South Americas, as well as in Africa, is characterised by febrile illness, frequent relapses and clinical complications such as severe anaemia. *P. vivax* relapse can only currently be prevented by primaquine (PQ), an 8-aminoquinoline which requires administration over 14 days. Tafenoquine (TQ) is a new 8-aminiquinoline anti-malarial drug being co-developed by GlaxoSmithKline and Medicines for Malaria Venture. TQ has been shown to be well tolerated in clinical studies in >4000 subjects, and possesses activity against all stages of the *Plasmodium* lifecycle including the dormant *P. vivax* hypnozoite. The DETECTIVE trial (Clinical Trial.gov identifier: NCT01376167) is a double-blind, randomised, parallel-group, active-controlled, seamless phase 2b/3 study. Part 1 (phase 2b) was conducted from Sep 2011 to Mar 2013 across 7 sites in 4 countries (Brazil, India, Thailand and Peru) to determine an efficacious and well tolerated dose of TQ to be co-administered with chloroquine (CQ) as radical cure for subjects with *P. vivax* malaria. 329 subjects (85 female; 244 male) who provided consent and met all entry criteria were randomised to one of six treatment arms: single dose 50, 100, 300, 600 mg TQ + CQ; CQ+PQ and CQ alone. Clinical and parasitological assessments were made on days 1, 2, 3, and on 10 subsequent follow-up visits until day 180. This part of the study was powered to detect a 30% difference between any of the doses of TQ+CQ and CQ alone in the primary endpoint of relapse efficacy 6 months post dosing. The secondary endpoints included relapse efficacy 4 months post dosing, time to relapse, parasite clearance time and fever clearance time. Key efficacy data from this study will be presented.

42B

A CLINICAL SUMMARY OF INVESTIGATIONS TO DETERMINE THE HAEMOLYTIC POTENTIAL OF TAFENOQUINE IN G6PD DEFICIENT SUBJECTS

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Tafenoquine (TQ) is an 8-aminoquinoline (8-AQ) in development as a single dose treatment for the radical cure of *Plasmodium vivax* malaria. However, 8-AQs cause haemolysis in glucose-6-phosphate dehydrogenase deficient (G6PDd) individuals. Although TQ will be contra-indicated in G6PDd individuals, it is important to know the risk associated with accidental dosing of these moderately G6PDd subjects (e.g. misclassified by G6PD tests or contra-indication is ignored). We present a summary of the haematological safety data from recently completed Ph1 and 2b studies. TAF110027, as previously reported, data demonstrate that healthy female volunteers (Hb >12 g/dL), heterozygous for the G6PD Mahidol mutation (40-60% G6PD activity, % of locally defined median value), dosed with primaquine 15 mg daily for between 9-14 days had a median maximum fall in Hb of 2.8 g/dL (n=4, range 2.1-3.0). A similar median fall in Hb of 2.8 g/dL (n=3, 2.7-3.0) was seen in subjects dosed with 300

mg TQ. The Ph2b DETECTIVE (TAF112582) dose ranging study enrolled 329 subjects with ≥70% G6PD activity and all females underwent G6PD gene sequencing. The maximum decline in Hb in any subject in this study within the first 14 days was 4.2 g/dL (range -4.2 to +5.8). Specifically data from G6PD deficient subjects from TAF110027 and TAF112582 will be presented with data from two G6PD heterozygous subjects (Vanua Lava enzymatic activity 8.8 IU/gHb, 81% and A- 11.0 IU/gHb, 102%) who were dosed with 300 and 600 mg TQ respectively in the TQT study, as reported previously. These individuals had Hb falls of 2.1 and 1.9 g/dL but remained clinically asymptomatic. Taken together these data inform on the safety and maximum tolerated TQ dose in heterozygous females and suggest that in subjects with ≥70% G6PD activity a single dose of ≤300 mg of TQ would be clinically tolerated in genetically G6PDd *P. vivax* patients. Thus should they be treated with TQ the Hb drop will be balanced by the positive benefit:risk to the patient of preventing further relapses of *P. vivax*.

43

BALANCING POTENCY AND TOXICITY: OPTIMIZATION OF ELQ-400 FOR SINGLE DOSE CURES IN MALARIA

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The eradication of malaria ultimately depends on the ability of antimalarial drugs to broadly and safely eliminate *Plasmodium* parasites within a population. Unfortunately, individuals at the greatest risk for malaria often live in remote regions with limited access to treatment and little ability to finance drug regimens. Even when drug therapy is available, there is a high risk of non-completion - leading to recrudescence, drug resistance, and an increased malaria burden within the community. The discovery of a potent, single-dose cure for malaria would avoid many of these problems, and is highly sought in the quest for eradication. Here, we describe the efforts to develop and optimize one such single-dose therapeutic, ELQ-400. ELQ-400 is a next-generation endochin-like quinolone (ELQ) with remarkable *in vitro* and *in vivo* potency against *Plasmodium* parasites. In a 4-day *in vivo* test against *P. yoelii* in CF1 mice, ELQ-400 exhibits an oral non-recrudescence dose of 0.1 mg/kg/day and a transdermal non-recrudescence dose of 1 mg/kg/day. More intriguingly, single dose oral cures can be obtained in this model with doses as low as 2 mg/kg. Mechanistically, ELQ-400 is a mitochondrial *bc1* complex inhibitor, with likely activity at the oxidative (Q_o) site exploited by atovaquone. Unfortunately, both ELQ-400 and atovaquone demonstrate mild off-target effects on the human *bc1* complex. The EC50 values against human HEK cell derived *bc1* are 0.2 μM and 0.86 μM for atovaquone and ELQ-400, respectively. This project describes our efforts to reduce this off-target activity and optimize ELQ-400 for human use. We have created a varied ELQ-400 analog library and determined the potency of each compound against *Plasmodium* parasites *in vitro* and *in vivo*. Parasite vs. host selectivity was then evaluated by comparing *bc1* complex inhibition in *Plasmodium* and HEK derived mitochondria. This project provides structure-activity insight that will allow us to balance single dose potency with acceptably low host toxicity, and ultimately create a single-dose antimalarial for human use.

44

INNOVATIVE TOOLS TO SELECT THE NEXT GENERATION OF ANTIMALARIAL DRUGS

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The reliability of current malaria treatment options is under continual threat from the spread of resistance with even the newest classes of antimalarials showing evidence of clinical failure. Consequently, there is a clear need for new medicines to replace those compromised by resistance as well as potentially identifying novel therapies that offer significant advantages over the current standard of care. To address these issues, the malaria scientific community has conducted several high scale phenotypic screens and thousands of starting points for antimalarial discovery have been identified. Following this initiative we created the Tres Cantos Antimalarial Set (TCAMS) that comprises of over 13K novel hit compounds that are freely available to the global drug discovery community. The challenge with such a rich source of chemical diversity is how to identify the most optimal molecules to enable the identification of differentiated new medicines. To aid with this process, the Malaria DPU has developed a range of innovative tools to support the efficient triaging of compounds from TCAMS and to find the most optimal starting points for drug discovery efforts. One of these tools is the *in vitro* parasite reduction rate assay (PRR) that can efficiently identify compounds that have a fast-acting mode of action and potentially enable the discovery of novel antimalarial to replace current front-line treatment options. A second approach has been the development of a gametocytocidal high throughput assay to identify dual acting compounds active against blood stages and with transmission blocking potential. Another innovative approach is the *P. berghei* mouse screening model which has been used successfully to prioritize hits directly from TCAMS that show robust *in vivo* efficacy and thus have intrinsic properties that should enable optimization through to an oral drug candidate. We describe how these tools have been successfully deployed to efficiently select tractable starting points to support new lead optimization efforts and potentially reduce drug discovery cycle times.

45

NOVEL ASSAYS FOR DRUGS AGAINST *PLASMODIUM FALCIPARUM* GAMETOCYTES AND GAMETES

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Targeting malaria transmission is increasingly recognized as a crucial step along the road towards malaria control and eradication. Nevertheless, with the exception of primaquine, no drugs are currently available to efficiently target *Plasmodium falciparum* gametocytes. An important reason for this major gap is that *in vitro* assays to screen for gametocytocidal activity of existing antimalarials and of libraries of new compounds are still suboptimal. In particular available assays still fail 1) to specifically measure compound inhibitory effects against different stages of gametocyte development and 2) to reliably monitor viability of the terminally differentiated mature gametocyte. Our work aimed to develop novel cell based assays amenable to be used as sensitive, robust and reliable M-HTS assays for gametocyte-blocking drugs. 1) In order to specifically assess activity of compounds against early vs late gametocytes we expressed for the first time in malaria parasites novel luciferase reporter genes with distinguishable emission properties under the control of sexual stage specific regulatory sequences switched on early and late in gametocytogenesis. In this work we screened for and identified novel *P. falciparum* gene regulatory sequences able to turn on expression of

reporter genes in mature gametocytes at a higher efficiency than the currently used late gametocyte promoter from gene *pfs28*. 2) In order to detect and measure viability of the mature gametocytes for novel anti-gamete assays, we developed an imaging based assay which quantitatively measure ability of stage V gametocytes to undergo the first step in the maturation into gametes. Both types of assays have been validated with described anti-gametocyte drugs and are now in the position to be used for wider screenings to measure ability of existing anti-asexual stage compounds to also inhibit gametocyte/gamete development as well as to identify novel transmission blocking compounds.

46

PRECLINICAL EVALUATION OF A POTENT NEW ANTIMALARIAL, JPC2997

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The emergence of artemisinin-resistant *Plasmodium falciparum* in western Cambodia and in south Vietnam highlights the urgent need to develop better antimalarial drugs. This study investigates a new Mannich base, JPC2997, with excellent *in vitro* and *in vivo* efficacy and low cytotoxicity. When tested *in vitro* against the *P. falciparum* chloroquine-sensitive D6 line and the chloroquine-resistant W2 line, mean IC_{50} values were 14 nM and 7 nM, respectively. Cytotoxicity was assessed against 3 mammalian cell lines; HepG2 (human hepatocarcinoma), HEK293 (transformed human embryonic kidney) and BHK (baby hamster kidney), with IC_{50} values >35 μM for each line. This equates to selectivity index >2500. To assess the *in vivo* suppression dose of JPC2997 in mice, the ED_{50} values following oral dosing were 0.54 mg/kg/day for JPC2997 compared with 1.1 mg/kg/day for chloroquine and 1.3 mg/kg/day for dihydroartemisinin in the Peters 4-day test using the *P. berghei* ANKA strain. The radical curative dose of JPC2997 was remarkably low at 4 mg/kg/day using the modified Thompson test compared with 128 mg/kg/day for both chloroquine and dihydroartemisinin. Preliminary pharmacokinetics of JPC2997 in mice and Aotus monkeys have shown the drug to be rapidly absorbed, with elimination half-lives of about 53 hours in mice and 8.5 days in Aotus monkeys. JPC2997 was found to have a blood to plasma ratio of approximately 0.7. With regards safety assessment, JPC2997 was found to be Ames test negative, have good microsomal stability and low hERG inhibition. JPC2997 analogs are also being investigated for improved potency and safety. Together these data suggest JPC2997 is an excellent candidate antimalarial and may be a suitable partner drug with an artemisinin derivative for the treatment of malaria.

47

A HUMANIZED MOUSE MODEL FOR TRANSMISSION BLOCKING ANTIMALARIAL ASSESSMENT AND DISCOVERY

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Antimalarial drugs that effectively kill the transmission stage of the malaria parasite lifecycle, the gametocyte, are recognized as key tools required for the global elimination of malaria. Currently only one drug, primaquine, is clinically available for the clearance of *Plasmodium falciparum* gametocyte stages, however the use of this drug is limited by toxicity. Therefore, the search for new antimalarials that can clear gametocytes is an important priority in malaria research. Unfortunately methods that enable the identification of potential anti-gametocyte agents in *in vivo* animal studies are limited. Animal studies are important as they enable the activity of metabolites to be assessed. *In vivo* methods for examining the activity of compounds against gametocytes rely on mouse models and murine *Plasmodium* species. While these models are useful, their relevance to human disease, particularly *falciparum* malaria is questionable due to significant differences in gametocyte development. To improve the current position we have developed a mouse model that enables *P. falciparum* gametocytes to be studied *in vivo*. Maintenance of the gametocytes within mice requires manipulation of the mouse immune system. Infection is established in SCID mice (deficient in B and T lymphocytes) that are administered liposomal clodronate to deplete macrophages. Clearance of parasites is also limited by removing the host's spleen and overwhelming the immune system with human erythrocytes prior to infection. Pure gametocyte cultures are FITC stained before injection and the kinetics of gametocyte clearance after drug treatment is assessed by fluorescence activated cell sorting. Humanized mouse models provide a novel and accessible means of anti-gametocyte antimalarial assessment and enable the important step of *in vivo* analysis prior to human trials in the drug development pipeline. The steps involved in the development of the model and data from the validation of the model using currently available antimalarials will be presented.

48

A DOSE RANGING STUDY IN THE HUMAN INDUCED BLOOD STAGE MALARIA MODEL TO DEFINE THE ANTIPARASITIC ACTIVITY AND PHARMACOKINETIC-PHARMACODYNAMIC RELATIONSHIP OF THE SYNTHETIC PEROXIDE ANTIMALARIAL OZ439

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The synthetic ozonide peroxide antimalarial OZ439 is under development by Medicines for Malaria (MMV), and has been designed to provide single-dose oral cure of malaria infection. Phase I and IIa clinical trials have demonstrated safety and promising activity. To define the activity of OZ439 in single dose among malaria-naïve subjects, a study was undertaken using the human induced blood stage *Plasmodium falciparum* infection model. Parasitemia was monitored by qPCR and drug levels measured by LCMS. Treatment was designated to begin once *parasitemia* reached a threshold of >1,000 parasites/mL. The drug was administered as a single dose of 100, 200 and 500 mg to 3 groups of 8 healthy volunteers. The drug failed to clear parasites in the two lower dose cohorts, with all 16 subjects requiring intervention with curative therapy with artemether/lumefantrine within 72 hours of administration of OZ439. The 500 mg dose resulted in rapid parasite clearance, with a PRR as determined by PCR of 4.0 (95% CI 3.8-4.3). During the 14 day followup phase, 4 of the 8 subjects in this cohort experienced recrudescent *parasitemia*, as determined by PCR, and required curative therapy with artemether/lumefantrine. Apart from transient symptoms of malaria, no clinically significant adverse events occurred among the 24 subjects. Modelling of the PK/PD relationship will be presented. This study demonstrates that a single dose of ≤500 mg OZ439 will require co-formulation with a partner drug or multiday therapy to reliably clear *P. falciparum parasitemia*.

49

PEDIATRIC PHARMACOVIGILANCE: USE OF PHARMACOVIGILANCE DATA MINING ALGORITHMS FOR SIGNAL DETECTION IN PHASE IIIB CLINICAL TRIALS SAFETY DATASET FROM 7 AFRICAN COUNTRIES

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Medication safety needs all drugs to be monitored for their entire market life because early detection of adverse drug reactions (ADRs) can lead to alerts that prevent harm in both paediatric and adult patients. Pharmacovigilance programmes monitor and help ensure the safe use of medicines which is critical to the success of public health programmes. The common method for discovering previously unknown safety issues during post-marketing is through spontaneous reports. This study examines the use of data mining algorithms to identify signals from adverse events reported in a phase IIIb clinical trial that evaluated the efficacy and safety of several artemisinin-based combination treatments (ACT) in African children. We used safety data from a randomized, open-label non-inferiority clinical trial conducted in 12 sites in seven countries. Each site compared three of four ACTs, namely amodiaquine-artesunate (ASAQ), dihydroartemisinin-piperaquine (DHAPQ), artemether-lumefantrine (AL) or chlorproguanil/dapsone and artesunate (CD+A). We applied and assessed two pharmacovigilance signals generation methods (proportional reporting ratio [PRR] and Bayesian Confidence Propagation Neural Network [BCPNN]).Overall, 4,116 children 6-59 months old with uncomplicated *Plasmodium falciparum* malaria were treated (1,226 to AL, 1,002 to ASAQ, 413 to CD+A and 1,475 to DHAPQ), actively followed up until day 28 and passively for the next six months. A total of 6,238 adverse events were reported, resulting into 346 drug-event combinations. Ten signals were identified where nine were generated by PRR and one more by BCPNN only. Review using manufacturer package leaflets/DoubleCheckMD and further by malarialogists reduced the signals to five that needed further detailed evaluation. These two data mining methods work well in predicting signals. Use of experts and resources like manufacturer package leaflets can be essential in reviewing signals. Phase IIIb clinical trial safety data can be used to supplement spontaneous reporting systems reports and to validate previously reported ADRs.

50

RATS AND RISK IN THE MEKONG DELTA: SEROPREVALENCE OF SELECTED ZOONOTIC VIRUSES IN PEOPLE INVOLVED IN THE RAT MEAT TRADE

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In Vietnam, rice field rats are frequently trapped and sold in wet markets for human consumption, and activities relating to the rat trade present potential hazards for public health. As part of a larger program of study on viral zoonoses and risk of pathogen emergence in Vietnam, we have established community-based field research sites in the Mekong Delta, involving longitudinal cohort studies of people involved in the rat trade and in smallholder mixed-species farming operations. In parallel, we have conducted field-trapping of small mammals in peridomestic and forest habitats (n=136), and cross-sectional surveys of rodents sold in wet markets (n=150, 10 markets, 5 provinces). Here we report seroprevalence data from humans (n=200) and eight species of rodents for five groups of rodent-borne viruses, namely hantaviruses (Seoul, Dobrava, and Hantaan serogroups); arenaviruses (LCMV serocomplex); flaviviruses (TBE serocomplex and Phnom Penh Bat virus from the No Known Vector group); poxviruses (Cowpox); and parechoviruses (Ljungan). Confirmatory genetic identification and characterization of viruses from rodent tissue extractions are ongoing, as is clinical follow-up of sporadic illness episodes occurring within cohort members. Data on pathogen prevalence in rodent reservoirs will be linked to community-based and hospital-based surveillance to enable inferences regarding the frequency of symptomatic and asymptomatic cross-species transmission events, as well as risk assessments and implications for public health.

51

HUMAN MONKEYPOX IN A CONFLICT REGION OF THE DEMOCRATIC REPUBLIC OF THE CONGO

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Monkeypox is a zoonotic *Orthopoxvirus* infection with a clinical presentation similar to smallpox. The average case fatality rate has been reported to be 11%. Monkeypox virus is endemic in areas of western and central Africa, with the vast majority of reported cases in the Democratic Republic of the Congo (DRC). Ecological niche models predict disease in many areas of DRC, but exclude much of North and South Kivu Provinces located in the eastern portion of the country. In 2011 and 2012, three human monkeypox cases were confirmed by laboratory diagnostics in North Kivu (2 cases) and South Kivu (1 case). Each of these patients had travelled from or presented to clinics in the western most areas of the provinces, areas included in predictive ecological niche models of disease occurrence. The investigation of one case in Butembo, North Kivu revealed the patient had recently travelled from a highly forested area and had eaten bush meat during his voyage. The investigation identified 30 contacts while the patient was ill and 23 were healthcare workers. Of these contacts, 16 (53%) had no prior vaccination against smallpox.

No contacts became ill and no additional cases were reported from neighboring health zones. These three cases are the first to be reported from the two Kivu provinces since one report in 1983. This is an area that is experiencing armed conflict and population displacements. There are an estimated 1.8 million internally displaced persons in North and South Kivu, with movement of people to more forested and less conflicted areas, but with greater risk of zoonotic infections such as monkeypox. Thus, there is a potential higher risk of disease occurrence and spread in humans due to population displacements. Education of health workers will be critical for clinical recognition and enhanced surveillance to better characterize disease in this region of the country

52

NIPAH VIRUS SHEDDING AMONG *PTEROPUS* BATS IN THE CONTEXT OF A HUMAN OUTBREAK IN BANGLADESH, 2012

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Nipah virus (NiV) causes fatal encephalitis in humans; *Pteropus* bats are the natural reservoir for NiV and shed the virus through urine and saliva. In Bangladesh, the major route of NiV transmission from bats to humans is through consumption of raw date palm sap contaminated by bats. During January 2012, two clusters consisting of 7 human NiV cases occurred following two common exposures of drinking raw date palm sap. Our study objective was to determine the duration of NiV shedding among *Pteropus* giganteus fruit bats living near the human cases. There were 5 active *Pteropus* bat roosts within a 10 kilometer radius of the NiV outbreak sites and we counted bat population using the branch estimation method. We made four field visits: on days 18-15, 34-37, 47-50, and 61-66 after the first common exposure to date palm sap. We collected pooled urine samples from 1 to 3 roosts each night by laying 4- to 6-foot wide polythene tarps directly beneath the branches where the bats roosted between 3 and 4 AM. We collected pooled bat urine samples from the tarps at dawn, put it in lysis buffer, and analyzed for the presence of NiV RNA by quantative RT-PCR. On average, roosts had 230 bats (range: ~100 - 450). Of the 436 pooled urine samples collected under the roosts, we identified NiV RNA in 22% (26/117) visit 1 samples, 2% (2/98) visit 2 samples, and 1% (1/120) visit 3 samples and 0% (0/101) visit 4 samples. We detected NiV RNA in urine samples from three roosts during visit 1, two roosts during visit 2, and in one roost during visit 3. We did not detect any shedding from the remaining two roosts, one of which was cut down by villagers during visit 2. This investigation identified *Pteropus* bats shedding NiV near human outbreak sites for two months following the probable human exposures. Sampling roosts near human outbreak sites during early weeks of the outbreak may be a good strategy to detect the virus from the natural reservoir. Inhabitants of districts where NiV outbreaks are identified and surrounding areas could be at a higher risk of contracting NiV, even months after the first cases are detected because of prolonged shedding. Behavior change communications aimed at discouraging consumption of raw date palm sap should be disseminated throughout the sap production season to limit further NiV cases.

53

EXPOSURE FACTORS AND DISEASE SYMPTOMS OF HUMAN RIFT VALLEY FEVER IN SANGAILU, KENYA

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Rift Valley fever virus (RVFV) causes an acute, mosquito-borne viral disease in livestock and humans. To determine the exposure factors and range of disease symptoms associated with human RVF, we performed a household cluster survey in six villages in Northeastern Kenya in 2011. 1081 participants were tested via anti-RVFV IgG ELISA, yielding 16% seroprevalence (95% C.I. 0.1-0.2). No significant differences were found among villages. 31% (154/498) of adults were seropositive vs. 3% of children (≤15 years; 17/583). With each additional year of age, participants were 5% more likely to be seropositive (95% C.I. 1.0-1.1). Documentation of a 3y/o seropositive boy confirmed interepidemic transmission. Males were 2.6 times more likely to be seropositive (p<0.001; 95% C.I. 1.7-3.8); herders were 1.7 times more likely (p=0.004, 95% C.I. 0.9-3.0); whereas those who reported killing animals were 3 times more likely (95% C.I. 1.8- 4.9). Those with backache history were 4.6 times more likely to be exposed (p<0.001, 95% C.I. 3.0-7.2); those reporting photophobia were 3.9 times more likely (p<0.001; 95% C.I. 2.5-6.1); those reporting meningismus were 3.3 times more likely (p=0.007; 95% C.I. 1.4-8.0); those with history of eye pain were 1.9 times more likely (p=0.003, 95% C.I. 1.2-2.9); and those reporting malaise were 1.8 times more likely (p=0.005, 95% C.I. 1.2-2.7). Participants with poor visual acuity (>6/9) on examination were 3.5 times more likely to be exposed (p<0.001, 95% C.I. 2.2-5.5). Confirmatory plaque reduction neutralization testing results are pending. Our results demonstrate that RVFV exposure remains common in Northeastern Kenya and continues to be transmitted during interepidemic periods. Older age, male gender, herder occupation, and killing livestock were associated with increased RVFV exposure. Poor visual acuity, photophobia, meningismus, and backache were highly correlated with RVFV seropositivity and may be useful signs of RVFV human transmission and disease in endemic settings.

54

THE GLOBAL DISTRIBUTION OF CRIMEAN CONGO HEMORRHAGIC FEVER

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The contemporary worldwide distribution of the risk of Crimean-Congo hemorrhagic fever (CCHF) is poorly defined, making allocation of resources and public health efforts problematic. Here we undertake an exhaustive assembly of known records of CCHF occurrence worldwide from 1961 to the present, and use a formal modelling framework to map the global distribution of CCHF risk. We do this by first deriving a consensus on country-level presence or absence, and combine this information with the locations of known occurrences and a suite of high spatial-resolution covariates related to climate, urbanisation, agriculture, and livestock presence to derive the probability of occurrence at a 5km x 5km resolution globally. We find CCHF to be confined to Africa, Eastern Europe, and western Asia, but with spatially heterogeneous levels of risk within these regions. Our new risk map provides novel insights into the global, regional and national threat posed by CCHF, and highlights the need for cohort studies to be carried out in high-risk zones in order to determine the public health burden posed by this neglected disease. We intend for our contemporary risk map to serve as a starting point for a wider discussion

about the global impact of CCHF, and for it to help guide improvements in drug and vector-control strategies as well as evaluation of the economic burden caused by this disease.

55

EASTERN EQUINE ENCEPHALITIS: FIRST DOCUMENTED EPIDEMIC IN LATIN AMERICA AND CO-CIRCULATION OF VENEZUELAN EQUINE ENCEPHALITIS

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Eastern (EEEV) and Venezuelan (VEEV) equine encephalitis viruses are pathogens of humans and equids in the Americas. However, outbreaks of human EEE have never been reported in Latin America. In North America human EEEV infections average only 5-6 per year. In Latin America, equine EEE is common, but only 3 human cases have been recognized. Outbreaks of neurologic disease in humans and horses were reported in Panama from May-July, 2010. Antibody assays, viral RNA detection and virus isolation were performed on hospitalized patient sera, and additional cases were detected with enhanced surveillance. Eighteen patients from Darien were hospitalized with encephalitis. Of these, 7 were confirmed as EEE, 3 as VEE, and 1 with EEEV/VEEV co-infection. In a total 25 cases were confirmed for alphaviral infection (included the hospitalized described above): 13 EEEV, 11 VEEV and one dual infection. Phylogenetic analyses placed 2 equine EEEV isolates within subtype III. The 2010 EEEV strains differed from each other by 19 nucleotides, and by 28-36 nucleotides and 4-10 amino acids from the 1984 and 1986 Panamanian isolates. A human VEEV occupied a subtype ID associated with prior human disease in Panama. None of these differences is known to affect virulence. The reason(s) for the sudden appearance of human EEE concurrent with VEE is unknown. Phylogenetic results ruled out the introduction of a new EEEV strain; other possible explanations include: 1) increased surveillance detected EEE cases; 2) increased exposure of people to EEEV due to ecologic changes or enhanced enzootic circulation, or; 3) increased virulence or altered host range of Panamanian EEEV. Although our phylogenetic studies indicated that the EEEV strains infecting horses, and presumably humans, descended from Panamanian strains circulating during the 1980s rather than recent introductions. A recent change in virulence or host range of EEEV in Panama also remains possible. Virulence and host range determinants of EEEV are poorly understood.

56

INHIBITION OF EASTERN EQUINE ENCEPHALITIS VIRUS REPLICATION BY A HOST MICRORNA DETERMINES TISSUE TROPISM AND SEVERITY OF ENCEPHALITIS *IN VIVO*

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Eastern equine encephalitis virus (EEEV) is a highly neurovirulent, mosquito-borne alphavirus that results in significant mortality rates in symptomatic individuals. EEEV infection is often characterized by a limited prodrome promoted by a combination of restricted virus access to lymphoid tissues due to heparan sulfate binding, and the inability of EEEV to replicate in myeloid cells. Previously, we demonstrated that EEEV fails to initiate myeloid cell replication due to a deficiency in translation of the viral genome. Using translation reporters encoding the 5′ and 3′ non-translated regions (NTR) of EEEV, we have mapped the block in translation in myeloid cells to the 3′ NTR. Transfer of the EEEV 3′ NTR to a host mimic mRNA translation reporter resulted in a similar block in translation in myeloid cells. Two microRNA prediction algorithms, miRanda and PITA, predicted multiple binding sites for the myeloid cell-specific microRNA, mir-142-3p, in the EEEV 3′ NTR, while other alphaviruses did not contain these sites. Transient expression of mir-142-3p in mesenchymal (BHK) cells prevented EEEV infection but not that of another alphavirus, Venezuelan equine encephalitis virus, compared to a control microRNA. Deletion of the mir-142-3p binding sites in the 3′ NTR of EEEV did not affect viral growth in BHK cells, but resulted in the rescue of myeloid cell infection in macrophages and dendritic cells *in vitro* and in the lymph nodes of CD-1 mice *in vivo*. Infection of CD-1 mice with the EEEV deletion mutant results in both increased prodrome and average survival time compared to WT EEEV. Interestingly, this deletion in the 3′ NTR of EEEV suppresses viral replication in C6/36 mosquito cells and may suggest a mechanism through which the binding sites are preserved during virus cycling in nature. The presence of mir-142-3p binding sites in the 3′NTR of EEEV may represent a novel mechanism to restrict cell tropism that may suppress innate immune responses.

57

WOLBACHIA INFECTION DELAYS EXTRINSIC INCUBATION PERIOD IN DENGUE INFECTED MOSQUITOES

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Dengue is the most prevalent arthropod-borne virus with roughly 40% of the world's population at risk of infection each year. *Wolbachia pipientis,* which is an obligate intracellular bacterium capable of spreading itself through populations by manipulating the reproduction of its hosts, has become a promising biocontrol strategy. The *Wolbachia* strain *w*Mel, which has been artificially introduced into the dengue virus mosquito vector *Aedes aegypti,* decreases the susceptibility of mosquitoes to dengue virus and was shown to invade and replace natural mosquito populations. Extrinsic incubation period, which is the viral incubation period between the time when a mosquito takes a dengue infected bloodmeal and the time when the mosquito is capable of infection another individual, is one of the key components of transmission. We devised a non-destructive method to repeatedly sample pools of dengue infected mosquitoes to measure an individual's EIP. We show here that the *w*Mel (1) delays EIP in dengue infected mosquitos by 1.1 day, (2) narrows the window of infectivity of infected mosquitoes by around 4 days and (3) reduces the amount of dengue copy number in mosquito saliva by approximately 3 fold. Surprisingly, we found that *w*Mel (4) lengthened the lifespan of dengue infected mosquitoes possibly by lessening their dengue viral burden. These findings allow us to measure the vectorial capacity of *Wolbachia* infected mosquitoes and more accurate estimate of the impact of releasing *Wolbachia* infected mosquitoes as a strategy to reduce dengue transmission.

58

FIELD DEPLOYMENT OF WMEL AND WMELPOP *WOLBACHIA* INFECTIONS IN *AEDES AEPGYTI* FIELD POPULATIONS TO BLOCK DENGUE TRANSMISSION IN AUSTRALIA AND VIETNAM

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Wolbachia is a very common intracellular bacterial infection of insects that is maternally inherited and present in up to 70% of all insect species. It does not occur naturally in the major insect vectors of disease however. Recently we have been able to transfer different strains of *Wolbachia* into *Aedes aegypti* where it is maintained and maternally transmitted between generations. It induces a number of effects in the mosquito host including a direct interference effect with dengue viruses, greatly reducing the ability of the mosquito to transmit virus. I will report recent results of field trials in Australia and Vietnam where 2 strains of *Wolbachia* have now been introduced into wild mosquito populations that demonstrate that this technology can be readily deployed as a sustainable and novel approach to dengue control.

59

GENOMIC FEATURES OF INDIVIDUAL CHROMOSOMES IN THE YELLOW FEVER MOSQUITO *AEDES AEGYPTI* REVEALED BY LOW RESOLUTION PHYSICAL MAPPING

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Widespread mosquito *Aedes aegypti* is the primary vector of the yellow fewer and dengue viruses. Among other mosquitoe species with sequenced genome, *Ae. aegypti* has the largest genome with the size of 1376 Mb. About 47% of its genome is represented by transposable elements (TEs). However, the distribution of various repetitive elements along the chromosomes of the mosquito remains unclear. Here we present mapping data for 449 BAC clones, which have been examined for their chromosome location. A total of 294 genomic scaffolds or 619 Mb of *Ae. aegypti* genome were assigned to the particular bands on chromosomes. This study developed a low resolution chromosome map for 45% of *Ae. aegypti* genome: 70 (23%); 142 (48%); and 82 (29%) genomic supercontigs were assigned to the chromosomes 1, 2, and 3, respectively. Supercontigs were not oriented or ordered within chromosome bands. Using bioinformatics we examined the distribution of protein-coding genes, TEs and satellite DNA in three chromosomes of the mosquito. Chromosome 1 had the lowest gene density of 10.07 per 1 Mb and highest content of satellites (6.6%) and TEs (1715.1 per 1 Mb). Chromosome 2 had intermediate gene (11.87 per 1 Mb) and satellite (4.79%) densities and the minimal number of TEs per 1 Mb (1579.06). These values for chromosome 3 were 12.85, 4.68%, and 1604.90, respectively. Centromeric regions in all chromosomes demonstrated lower gene densities and higher content of satellites and TEs. These regions usually form small heterochromatic blocks on all three chromosomes. In addition, region 1q21-1q22 of chromosome 1, which is also characterized by bright staining with YOYO-1 iodide, demonstrated higher densities of satellites and TEs. We considered these 4 regions to be heterochromatin. Currently, the general picture of the distribution of genes, satellites and TEs is rather homogenous among the chromosomes. It does not display any extremely high peaks and low valleys. More detailed physical mapping is required for the better understanding of the relationship between DNA content and chromosomal banding patterns in chromosomes of *Ae. aegypti*. This information will contribute to our more complete understanding of the genome organization and function in the yellow fewer mosquito.

60

GENE FLOW BETWEEN *AEDES AEGYPTI AEGYPTI* AND *AEDES AEGYPTI FORMOSUS* VARIES AMONG GENOME REGIONS

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Aedes aegypti is the major vector of the Dengue and Yellow Fever flaviviruses. The subspecies *Aedes aegypti aegypti* (Aaa) has a global distribution while *Aedes aegypti formosus* (Aaf) is limited to Sub-Saharan Africa. These two subspecies differ in flavivirus vector competence, which is a partially genetically controlled trait. Diversity within vector competence associated genes can be maintained by reduced gene flow. To identify genome regions with reduced gene flow, single nucleotide polymorphisms (SNPs) were identified by whole genome resequencing on two replicates of 25 field collected individuals from Thailand, Mexico, and 2 locations in Senegal using the Illumina platform. Sequence libraries were aligned to the 18,769 VectorBase transcripts, including introns, of known chromosomal location (301 x 106 nucleotides). LOD scores between populations were corrected for by subtracting LOD differences between replicates. We identified 23 regions across all three chromosomes associated with large genetic distances between Aaa from Thailand or Mexico and Aaf from Senegal. We also identified regions of genetic distance between males and females from Senegal, but the average LOD score between males and females was half that compared to between Senegal and Thailand. In addition, we sequenced 90 genes of known chromosomal location in 10 field collected individuals from Senegal (Aaf), and 10 field collected individuals worldwide (Aaa). Regions of reduced gene flow across all three chromosomes between Aaa and Aaf were identified by calculating Fst values, sequence divergence (dxy), nucleotide diversity (π), and dN/dS with the program DNASp. These results suggest that the amount of gene flow between Aaa and Aaf is not uniform, but instead clustered in specific genome regions. Genome regions with reduced gene flow between Aaa and Aaf could be maintaining differences in vector competence phenotypes.

61

GENETIC DIVERSITY AND LINKAGE DISEQUILIBRIUM IN THE *ANOPHELES ARABIENSIS* GENOME, AS REVEALED BY WHOLE GENOME SEQUENCING

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Anopheles gambiae s.s. is frequently referred to as the most important vector of malaria in Africa. As such it has been the main focus of malaria vector research and thus we have a considerable knowledge about the ecology and genetics of this species, including a detailed understanding of patterns of population subdivision and gene flow, insecticide resistance, and habitat preferences. However, there is growing evidence that the sister species, An. *arabiensis*, outcompetes *An. gambiae* s.s to become the dominant vector species in areas of high insecticide treated net (ITN) coverage. Consequently, there is a growing need for research into the ecology and genetics of this somewhat neglected vector so that future vector control scenarios can be planned and implemented effectively. Here we conducted whole genome sequencing on 24 An. *arabiensis* samples, so to further our understanding of patterns of genetic diversity and linkage disequilibrium (LD) in this species. In total, we found high levels of genetic diversity within the An. *arabiensis* genome, with ~800,000 high confidence single nucleotide polymorphisms (SNPs) detected. However, levels of SNP diversity were shown to vary significantly both within and between chromosomes, with lower diversity exhibited on the X chromosome, within some inversions and near the centromeres. This pattern is consistent with findings in the *An. gambiae s.s.* genome. Linkage disequilibrium within An. *arabiensis* was found to decay very rapidly across all chromosomes. Nonetheless, elevated LD was observed within some non-fixed inversions, suggesting that recombination is suppressed/reduced in those regions. Overall, however, the low levels of LD suggests that association studies in this taxon will be challenging for all but variants of large effect, and will require very large samples.

62

INFLUENCE OF DIURNAL TEMPERATURE FLUCTUATION ON SELECT *AEDES AEGYPTI* LIFE HISTORY TRAITS: IMPLICATIONS FOR VECTORIAL CAPACITY

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Mosquito breeding sites are continually exposed to the vagaries of changing climactic conditions. This includes exposure to changing mean temperature caused by cyclical climactic shifts, such as the El-Nino Southern Oscillation, as well as short-term cyclical changes in ambient temperature caused by solar warming during the day and radiative cooling at night. It can be argued that short-term daily fluctuations in temperature can influence mosquito life history traits much more drastically than shifts in long-term mean ambient temperature, whether over the course of weeks, months, or years. The majority of work to date has focused on the role of static mean temperatures in estimating life history traits. This propensity to focus on mean temperature for defining the thermal environment extends to the use of epidemiological models for examining variables and predictive outcomes of vector-pathogen systems. If field exposure to fluctuating temperatures during larval development can influence life history trait expression, then utilizing mean temperature as a parameter in any model may under or overestimate the degree of vectorial capacity for a given population. In this study we set out to examine the influence of diurnal temperature fluctuations during larval development on selected adult life history traits. Four cohorts of the dengue vector, *Aedes aegypti*, from two geographically separate areas (Belize and Thailand) were exposed as larvae to one of four diurnal temperature range (DTR) treatments from 0°C to 20°C around a daily mean of 28°C. Results suggest that larval exposure to diurnal temperature fluctuations influences the outcome of epidemiologically relevant life history traits and that these outcomes vary depending on the size of the DTR. Implications for vectorial capacity estimates are discussed.

63

HUMAN BLOOD FEEDING PATTERNS OF *AEDES AEGYPTI*: IMPLICATIONS FOR DENGUE EPIDEMIOLOGY

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Understanding how mosquito feeding patterns are allocated among different people can improve our understanding of the entomological processes that support pathogen transmission and may reveal targets for minimizing risk and breaking the pathogen transmission cycle. We used DNA profiling of human blood meals in the dengue vector *Aedes aegypti* to quantify its contact with human hosts and to infer the epidemiologic implications of its blood feeding behavior. We examined the number of different people bitten within a specified time period, whether biting frequency was related to host and mosquito age, host size, and the number of times each person in the community was bitten. An estimated 43-48% of engorged mosquitoes bit more than one person over consecutive days within a gonotrophic cycle. The majority of multiple blood meals during the dry and rainy seasons were from residents of the house where the mosquito was collected. Non-residents in both multiple and single blood meals lived in adjacent houses. In general, people under 25 years of age were bitten less frequently than older people. The majority of blood meals were from single hosts that were only detected in mosquitoes once or twice, but some hosts were bitten up to 14 times. Interaction networks for mosquitoes and human hosts revealed biologically significant blood feeding hot spots, including houses that functioned as local markets, representing community gathering places.

64

AMERICAN HISTOPLASMOSIS IN HIV-INFECTED PATIENTS: A STUDY OF PROGNOSTIC FACTORS ASSOCIATED WITH EARLY DEATH

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American histoplasmosis is a endemic fungal infection in French Guiana. In persons with AIDS, it is the most frequent opportunistic infection and the leading cause of death. In order to reduce deaths, it is important to identify prognostic factors associated with early mortality so that appropriate therapy can be given. We looked at a one of the largest series of patients available to determine risk factors for early mortality. A retrospective study was conducted to identify persons with HIV/AIDS infected with *Histoplasma capsulatum* var. *capsulatum* and admitted to one of the three main hospitals of French Guiana between 1992 and 2011. Early mortality was defined by death occurring within 30 days after antifungal treatment initiation. Data were collected on standardized case report forms and analysed using multivariable logistic regression models. A total of 274 patients with HIV/AIDS were identified with histoplasmosis during 1992-2011. Forty six patients met the criteria for early death. The final multivariate model found several factors associated with an increased risk of early death: dyspnea OR=11.36 [4.28-30.17], acute renal failure OR=7.23 [1.47-35.71], WHO performance status score > 2 OR=4.05 [1.86-8.82] and platelet count ≤ 100 000/mm3 OR=3.51 [1.34-9.16]. Cases found during 2005-2011, OR=0.02 [0.01-0.12], and those from Cayenne General Hospital, OR=0.13 [0.04-0.47], were associated with a reduced risk of early death. This is the largest case series looking at factors associated with early death for histoplasmosis, and for the first time after adjusting for CD4 counts. These results are consistent with other reports from the Americas. The factors identified can provide clinicians arguments about early and aggressive intervention with antifungal therapy in order to prevent early death due to histoplasmosis.

65

GENDER DIFFERENCES IN HIV DISEASE PROGRESSION AND OUTCOMES AMONG HIV PATIENTS ONE YEAR AFTER STARTING ANTIRETROVIRAL TREATMENT (ART) IN DAR ES SALAAM, TANZANIA

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The progression rates to AIDS associated with HIV infection might differ between women and men because of biological and socioeconomic factors. We conducted a study, in hospital setting in Tanzania, to assess the clinical, social demographic, virological and immunological differences by gender at the starting of antiretroviral treatment (ART) and outcomes after one year. A cohort study involving Adult HIV infected patients scheduled to start ART and followed up to 1 year on ART. Structured questionnaire and patients file review were used to collect patients' information and blood was collected for CD4 and viral load testing. Gender differences were assessed using Kruskal-Wallis test and chi-square test for continuous and categorical data respectively. Survival distributions for male and female patients were estimated using the Kaplan-Meier method and compared using Cox proportional hazards models. A total of 234 patients, 70% females were recruited. At baseline, women had a significantly lower education level; lower monthly income, lower knowledge on ARV, less

advanced HIV disease, (female's median CD4 count: 149; male's median CD4 count; 102) and higher BMI (female's 22: male's 20). After 1 year follow up, more females survived and had undetectable plasma viral load (females 69%; males 45%), worse CD4 cell increase and higher BMI (female 24.5; males 22.5). The unadjusted relative hazard for death for men compared to women was 1.94. After correcting for confounding factors, the Cox proportional hazards showed no significant difference in the survival rate. We observed differences in mortality in adult women compared to men on ART, despite of lower socioeconomical status and worse virological and immunological treatment response. This is due at least in part to the fact that women started treatment at a less advanced disease stage. We recommend continuous follow up of this and more cohorts of patients to better understand the underlying causes for these differences and whether this will translate also in longer term differences.

66

MANAGING ANTITRETROVIRAL THERAPY IN URBAN AND CAMP REFUGEE SETTINGS: CHALLENGES IN MONITORING ADHERENCE AND VIROLOGIC OUTCOMES

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Given the stresses of forced displacement, our objective was to compare adherence and virologic outcomes among refugees and surrounding host communities receiving antiretroviral therapy from shared clinics and to propose recommendations to promote consistent treatment success over time. Cross-sectional surveys were conducted among HAART clients (≥18 years) in Kuala Lumpur, Malaysia (urban) and Kakuma, Kenya (camp) using a structured questionnaire, a pharmacy-based measure (Rx) of HAART prescription refills over 24m prior to study start, and HIV viral loads (a confirmatory VL was used among clients initially unsuppressed in Kakuma). In Malaysia, 90% of eligible refugees (n=153) appearing on a UNHCR database and 81% (n=148) of serially-recruited host community clients participated. Similar proportions of those on treatment for ≥25wks from both groups achieved viral suppression (81% v. 84%, p=0.54) and optimal adherence (Rx, 74% v.66%, p=0.15; one-month recall, 72% v. 70%, p=0.79). Refugee status was not independently associated with the outcome (aOR=1.62, 95%CI 0.64-4.09;p=0.31). In Kenya, 85% (n=73) and 86% (n=86), respectively, participated; similar proportions of refugee and host clients on treatment for ≥25wks were not virologically suppressed (88% v. 89%, p=0.92). The proportion adhering to pharmacy claim schedule was 79% overall (85% v. 75%, p=0.14). In multivariable analyses, refugee status was not independently associated with unsuppressed viral load. A longer time since HIV diagnosis (aOR=6.81, 95%CI 1.20-38.58, p=0.02) and ≥8 household members (aOR=0.10, 95%CI 0.02-0.55, p=0.01) were independent risk factors. In summary, no differences in virologic outcomes were detected between refugee and host clients; however, levels of viral suppression were very low in the camp setting. Our findings support a policy of equal HAART provision and support, demonstrate the effectiveness of virologic measures, and underscore the necessity of valid and routine adherence monitoring for evaluating program effectiveness among refugees and other forcibly displaced populations, especially those based in remote camp settings.

67

PREDICTION AND EPIDEMIOLOGY OF THE IMMUNE RECONSTITUTION INFLAMMATORY SYNDROME IN GABON

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Immune Reconstitution Inflammatory Syndrome (IRIS) is defined as the paradoxical worsening of a pre-existing infection or presentation of a previously undiagnosed condition in HIV infected patients soon after initiating antiretroviral therapy (ART). IRIS is reported to be a frequent problem in patients starting ART. Not much is known about the epidemiology and predictive factors of IRIS in the Central African region. Due to the sero-diversity of HIV in the Central African region, incidence and clinical appearance of IRIS might be different to that in East or Southern Africa, where most research on IRIS has been done. We report on an on-going observational cohort study with a nested case-control design. All ART naïve patients starting ART at the HIV clinic in Lambaréné, Gabon, are asked to participate. Participants undergo a thorough clinical evaluation (history, physical exam, ultrasound, X-ray, visual capacity and fundoscopy) at baseline and are followed at week 2, month 1, 3, 6, 9 and 12 after initiation of ART. Plasma samples are stored for immunological analyses. Epidemiological data on the different types of IRIS and its elliciting pathogens are reported. A nested case-control study will provide insight into the pathogenesis of IRIS and may identify predictors for the syndrome. At the moment of writing 205 patients have been pre-screened and 56 patients have been included in the study. Patients are predominantly female (35/56, 62.5%) with a mean age of 39 years (SD 8.9 years). Baseline CD4 count is 186 cells/mm^3 (SD 144), and the most important opportunistic infection is tuberculosis (13/56, 23%). Incidence of IRIS is lower than expected with now 3 cases having developed a suspected IRIS (one patient developed an unmasking CMV uveitis, one an unmasking oral candidiasis, and one patient developed an unmasking Kaposi's Sarcoma). In conclusion, Incidence of IRIS in Gabon appears to be much lower than in other areas in sub Saharan Africa. Further analyses of possible explanations for this apparently low incidence will be part of this study. Inclusion is still on-going.

68

ACCEPTABILITY AND USE OF READY-TO-USE SUPPLEMENTARY FOOD COMPARED TO CORN-SOY BLEND AS A TARGETED RATION IN AN HIV PROGRAM IN RURAL HAITI

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There is no widely accepted consensus on the optimal composition, duration, or delivery mechanism of food assistance for patients with HIV. Ready-to-use supplementary food (RUSF) is increasingly used as a component of food rations, but its acceptability as a supplementary food and its intra-household distribution have not been extensively evaluated in adults. This qualitative study was embedded in a quantitative study comparing the impacts of RUSF and corn-soy blend on HIV program outcomes in rural Haiti. We evaluated the acceptability, sharing and use of the two food rations. Nine focus groups were conducted with 74 participants, 39 randomly selected from the RUSF arm of the study and 35 from the corn-soy blend arm. Focus groups were conducted using a guide with pre-designated core topics and open-ended questions. Data were recorded, translated, and synthesized into major themes. Four major themes emerged: ration taste, sharing of rations within the household, sharing with neighbors, and selling of rations. Preliminary results show that for recipients of both RUSF and corn-soy blend: all participants shared rations with household members; most participants reported that

children in their households consumed the largest portion of the rations; most participants shared rations with neighbors; and few participants reported selling or exchanging food rations. Most participants disliked the taste of corn-soy blend and almost all participants added it to the communal household food supply. RUSF was universally considered to be delicious and was frequently separated from the household food supply--participants often reserved a portion for their own consumption. In conclusion, RUSF was highly acceptable and was more often reserved for use by the individual with HIV in the household compared to corn-soy blend. Further evaluation of the intra-household use of food rations is critical to improving the efficacy of food assistance for patients with HIV who live in food-insecure settings.

69

PERFORMANCE OF A NEW GUIDELINE FOR ASSESSMENT AND MANAGEMENT OF ANEMIA IN HIV-INFECTED MOZAMBICAN ADULTS: FINDINGS FROM A PROSPECTIVE OBSERVATIONAL STUDY

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Anemia is associated with elevated mortality in HIV-infected adults. In Mozambique, presumptive treatment for malaria, intestinal helminths, and iron deficiency was once the standard for anemia care. In 2009, Mozambique's Ministry of Health disseminated a new guideline for management of anemia in HIV-infected patients attended by non-physician clinicians. The guideline requires clinicians to confirm anemia with a measured hemoglobin (Hb) level, and to expand the differential diagnosis to include tuberculosis (TB), adverse drug reaction (ADR), and WHO HIV Stage III anemia. One aim of our observational study was to describe the proportion of patients whose anemia could be managed successfully through use of the new guideline. In 2012 (April-September), we enrolled 324 ambulatory HIV-infected adults with measured Hb <10 g/dL (median age 29 years; 80.9% female [27.0% pregnant]; 30.1% on antiretroviral therapy [ART]; 74.0% on prophylactic co-trimoxazole; median Hb 8.8 g/dL; median CD4 T-lymphocyte count 279 cells/μL). Predefined study endpoints were reached by 199 (61.4%): 169 (52.2%) improved as confirmed by Hb increase of >=1 g/dL; 30 (7.4%) died or were hospitalized. The other 125 (38.6%) were lost to follow-up. Of the 169 who improved, 123 (72.8%) had no specific anemia treatment other than ferrous sulfate, folic acid, and anthelminthics (all), and antimalarials if indicated by positive test for *Plasmodium falciparum parasitemia* (35; 20.7%). Other interventions associated with improvement in the remaining 46 patients included initiation or continuation of treatment for TB (18 [10.7%]), discontinuation of zidovudine and/or co-trimoxazole for presumed ADR (20 [11.8%]), initiation of ART (13 [7.7%]), and treatment of laboratory-confirmed *bacteremia* (6 [3.6%]). These findings support the inclusion of an expanded differential diagnosis (including TB, ADR, and untreated AIDS) in Mozambique's new guidelines for non-physician clinicians who manage anemia in HIV-infected adults. In the future, inclusion of severe bacterial infections may also be of benefit.

70

GENOTYPES OF *TOXOPLASMA GONDII* INFECTING HIV/AIDS INDIVIDUALS AND HEALTHY BLOOD DONORS IN ACCRA, GHANA

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Toxoplasmosis has severe to fatal consequences in immunocompromised individuals such as HIV/AIDS clients. The parasite, *Toxoplasma gondii,* has three clonal types I, II and III, linked to different clinical outcomes, that is, asymptomatic, benign, or severe infection in healthy and immunocompromised humans. In developed countries, research has led to proper management and prevention of toxoplasmosis. In Ghana the extent of *Toxoplasma* infection in humans is, relatively, unknown. This study aimed at detecting and genotyping *T. gondii* clones in healthy blood donors and HIV/AIDS clients in Accra. This cross sectional study was conducted among attendants at the Korle-Bu Teaching Hospital from May to December 2011. It involved 148 HIV/AIDS patients (98 Females; 50 Males: 15 to 82 years; mean=40.11; Std. Dev. 10.25 years) and 149 healthy blood donors (18 Females; 131 Males: 19 to 94 years; mean=35; Std. dev. 14.23). Informed consent was obtained from pre anti-retroviral therapy HIV-positive individuals with 0≥CD4$^+$T-cell count/mm^3 blood≤200, and healthy blood donors. Genomic DNA was extracted from whole blood samples of participants using DNeasy® blood and tissue kit (QIAgen, USA). Nested PCR and restriction fragment length polymorphism analysis were employed to detect and genotype *T. gondii* using SAG3 and GRA6 markers. Data was analyzed using SPSS version 17. Overall, 54.7% (81/148) HIV-positive samples were positive for SAG3 and/or GRA6 *T. gondii* markers. Overall, 93.8% (76/81) positives were of clonal type II, 1.2% (1) type I, 1.2% (1) both type I and II whiles the genotype of 3.7% (3/81) could not be determined. For blood donors, 3.4% (5/149) were positive for the markers and 2 were type I and 3, type II. No type III was detected. There was a high prevalence of *T. gondii* clonal type II in both HIV-positive and healthy individuals. This indicates more animal source infections among the participants. This information may be helpful in the effective management of *T. gondii* infections in HIV/AIDS clients and the general prevention and control of toxoplasmosis in Ghana.

71

EXTANT CRYPTIC SEXUALITY IN *TRYPANOSOMA CRUZI* DRIVES THE EMERGENCE OF NOVEL STRAINS WITH EPIDEMIOLOGICALLY IMPORTANT PHENOTYPES

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Clonal propagation is considered to be the predominant mode of reproduction among many parasitic protozoa. However, this assumption may overlook unorthodox, infrequent or cryptic sexuality. *Trypanosoma cruzi*, the causative agent of Chagas disease, is known to undergo non-Mendelian recombination *in vitro*, while two of the six major circulating genetic lineages (TcV and TcVI) resemble meiotic F1 progeny. Despite the existence of natural hybrid strains, a pervasive view is

that recombination was an evolutionarily ancient phenomenon and contemporary genetic exchange is of little epidemiological relevance. We undertook high resolution nuclear and mitochondrial genotyping of field isolates belonging to TcI (n=300) and TcIV (n=80), the principal lineages responsible for human Chagas disease in northern South America. Gross nuclear-mitochondrial phylogenetic incongruence was observed at multiple levels, including among disparate populations as well as major lineages. In all cases, hybrids had undergone mitochondrial introgression without apparent reciprocal nuclear recombination between parental genotypes, implying additional, as yet uncharacterized, cellular mechanisms may facilitate natural hybridization in *T. cruzi*. In parallel, we performed *in vitro* phenotyping of recombinant strains to provide the first evidence that genetic exchange in *T. cruzi* is associated with altered axenic growth rates, mammalian cell infectivity and drug susceptibility. Together these results indicate that recombination is geographically widespread and continues to influence natural parasite population structures, driving the emergence of novel strains with epidemiologically important virulence traits, and challenging the traditional paradigm of clonality in *T. cruzi*. We describe current work elucidating the frequency of hybridization within an endemic disease focus in North East Colombia and along an ecological cline in Bolivia and discuss the implications of parasite sexuality for Chagas disease control in sylvatic and domestic transmission settings.

72

A CYTOKINE EXPRESSED BY *LEISHMANIA* REGULATES THE IMMUNE RESPONSE TO PROMOTE PARASITE PERSISTENCE

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Parasites of the genus *Leishmania* may disrupt the mammalian immune response, contributing to disease with symptoms ranging from scarring cutaneous lesions to systemic illness and death. Many species of *Leishmania* that infect humans produce orthologs of the cytokine macrophage migration inhibitory factor (MIF). To determine a role for this parasite-encoded MIF, a mutant strain of *L. major* was created in which the two MIF orthologs, Lm1740MIF and Lm1750MIF, were deleted by targeted gene replacement. This mif -/- *L. major* strain grew normally in culture and infected mouse macrophages at an equivalent rate to wild type L. major. However, the mif -/- *L. major* mutant was more susceptible to destruction by activated macrophages and it failed to prevent the activation-induced apoptosis of these cells when compared to wild type L. major. These phenotypic differences were dependent on host cell expression of the MIF receptor CD74, as revealed by studies in Cd74-/- macrophages. Whole genome microarray analysis demonstrated that a number of genes were down-regulated in macrophages infected with mif -/- versus wild type L. major, including inflammatory cytokines, chemokines and co-stimulatory molecules such as CD86 and ICAM-1. It was further determined that macrophages infected with mif -/- *L. major* were restricted in their maturation and were less proficient at presenting parasite antigen to T cell hybridomas bearing a *Leishmania* antigen-specific TCR. Finally, mif -/- *L. major* infection of BALB/c mice was associated with reduced cutaneous lesion size and parasite burden when compared to wild type parasites. Notably, T helper cells from mice infected with mif -/- *L. major* were skewed toward a long-lived, memory phenotype. These findings suggest a role for *L. major*-encoded MIF in modulating the host innate and adaptive immune response to promote parasite survival.

73

CD4+ T CELLS RELEASE *LEISHMANIA* SPECIFIC IFNΓ, THAT LIMIT PARASITE REPLICATION IN PATIENTS WITH VISCERAL LEISHMANIASIS

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Visceral Leishmaniasis (VL) is a fatal chronic disease caused by protozoan parasite *Leishmania* and characterized by prolonged fever, spleno-hepatomegaly, pancytopenia and leads to death if left untreated. One of the key immunological features of VL patients is that their peripheral blood mononuclear cells (PBMCs) do not proliferate and produce interferon-gamma (IFNγ) and/or IL-10 in response to leishmanial antigen. Employing a whole blood assay (WBA), we have recently reported, the surprising result that active VL patients secrete significant levels of IFNγ in response to soluble *Leishmania donovani* antigen (SLA). In the present study, we addressed the cellular source and the factor behind the antigen driven IFNγ in stimulated whole blood and effect of endogenous IFNγ on parasite replication. By, depleting different cell populations using whole blood magnetic columns and magnetic beads, CD4+ cells were found to be crucial for the IFNγ production. Intracellular staining and flow cytometric analysis confirmed CD4 T cells as the main source of *Leishmania* specific IFNγ in the WBA. We found that complements, antibodies and RBCs presents in whole blood do not play a significant role in the IFNγ response, while removal of CD15+ (neutrophils) and CD56+ (NK cells) reduced to the gamma production, suggesting that these cells also contribute to SLA induced IFNγ in WBA. Blockade of IFNγ in ex-vivo splenic aspirate cultures demonstrate that the endogenous IFNγ detectable in patients with VL limit parasite growth. Together our results suggest that antigen specific CD4 T cells producing IFNγ are present in patients with VL and that they serve to slow down parasite propagation.

74

THE CELLULAR EGRESS PROCESS OF *TRYPANOSOMA CRUZI*: A KEY, BUT NEGLECTED LIFE CYCLE EVENT OF THE CAUSATIVE AGENT OF CHAGAS DISEASE

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The intracellular protozoan parasite *Trypanosoma cruzi* is the causative agent of Chagas disease, the most important parasitic disease in Latin America. In mammalian hosts, including humans, the parasite preferentially invades endothelial, muscle and cardiac cells, where it multiplies. At the end of this replicative cycle, the parasite egresses from host cells through events which remain virtually unexplored. The host cell is destroyed in the process. *T. cruzi*'s cellular egress holds potential as target for chemotherapeutic intervention for Chagas disease, for which no vaccine or satisfactory chemotherapeutic treatment are currently available. Furthermore, understanding the process of host cell destruction is fundamental to clarify the pathogenesis of Chagas disease, which is still poorly understood. We aimed to perform an initial characterization of the cellular egress process of *T. cruzi*. We have developed a colorimetric assay to measure the parasite egress *in vitro*, employing the Tulahuén strain holding the β-galactosidase gene, which allows for monitoring the relative quantities of intra and extracellular (egressed) parasites in the late stages of infection of cells *in vitro*. Using this essay, we have tested several kinds of protease inhibitors and determined that the inhibitors of cysteine proteases (antipain) and metalloproteases (phosphoramidon) are able to partially inhibit the egress of the parasite by affecting trypomastigote motility. Furthermore, employing fluorescence microscopy, we identified marked structural alterations in the actin filaments of the host cells during the late stages of the intracellular cycle of the parasite. Overall, our data suggest that the parasite´s intracellular cycle has an impact over

cytoskeletal structure, confirms that cysteine proteases play a role in the egress process, and suggest that metalloproteases are also involved. Our results constitute an important initial approximation to the cellular events involved in *T. cruzi*'s egress from infected cells.

75

HOUSEHOLD COST OF TREATING FEVERS IN GHANA

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The burden of malaria seems to be reducing globally but sub-Saharan African countries continue to bear the greater burden of the disease considering the economic burden on the households. Malaria continues to be the number one cause of morbidity and mortality in Ghana. The burden of malaria can be felt both in terms of the direct costs of seeking treatment as well as the indirect costs of reduced household productivity. It is a cross sectional cost-of-illness study and it employs quantitative analysis. The study used self-reported fever as an indicator of malaria. The study sample was drawn from the entire Health and Demographic Surveillance System (HDSS) databases of the Dangme West, Kintampo and Kassena-Nankana districts which identified households eligible for an in-depth interview from August 2009 to June 2011. All patients from such households that have a history of fever in the previous two weeks were investigated with regard to the care seeking, the provider, the treatment received and the related costs. Our study observed that average direct OPD cost of treating fevers was GH¢16.54 ($11.25) and that the average cost of self-treatment is 5 times less than seeking care at OPDs of health facilities in Ghana. A household in Ghana is likely to pay GH¢31.43 ($21.38) as direct cost per episode of fever treatment, that is, 32.7% of monthly minimum income in Ghana. Government of Ghana in its effort to keep the direct cost of treating fevers relatively low through the provision of Health Insurance Scheme and the introduction of a subsidized AMFm drugs, the overall loss of productivity to the patients play a significant role especially when there are multiple fever cases within households in a year.

76

CHANGING INFORMAL PROVIDERS' DISPENSING BEHAVIOR IN UTTAR PRADESH, INDIA

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The state of Uttar Pradesh (UP) has the highest burden of diarrhea-related child mortality in India. Overall, 83% of caregivers seeking care for their sick child go to a private "doctor," who is often an informal, unqualified provider. Such providers are not recognized by the government, or by medical associations, and are shunned by pharmaceutical companies that have limited interest in covering poor, remote rural areas. Consequently, informal providers are "on their own," to provide sub-standard and potentially inappropriate or harmful care. The Diarrhea Alleviation through ORS and Zinc treatment (DAZT) project, supported by the Gates Foundation through the US Fund for UNICEF, has created an innovative partnership linking reluctant pharmaceutical companies and local NGOs to target the informal providers and change their diarrhea treatment behavior to dispense appropriate ORS and zinc treatment instead of antidiarrheal agents and antibiotics. Local NGOs provide a unique advantage: they are able to identify the providers and their representatives are welcomed by the suspicious providers. Pharmaceutical distributors ensure a consistent supply of drugs that is sustainable because of market forces. The DAZT intervention regularly covers 21,000 providers and drug sellers in 12 districts of UP and relies mostly on NGO field workers trained as medical representatives who call on the providers on a monthly basis and try to influence their prescribing behaviors. The project collects data daily through the field workers' cellphones and obtains detailed information on each provider through daily reports. An independent tracking survey performed on a quarterly basis is used to verify the results. The first survey round in March 2013 indicated that 38% of informal providers treated childhood diarrhea with ORS and zinc, 44% treated with ORS without zinc, and 4% treated with zinc without ORS. The results are discussed with the pharmaceutical and NGO partners to increase the efficiency of the intervention through ensuring a stable supply of ORS and zinc to the regular users and target future marketing efforts on non-users.

77

PRELIMINARY RESULTS OF A SYSTEMATIC REVIEW OF THE EFFECTIVENESS OF STRATEGIES TO IMPROVE HEALTH WORKER PERFORMANCE IN LOW- AND MIDDLE-INCOME COUNTRIES

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Health workers (HWs) play key roles in delivering health interventions. In low- and middle-income countries (LMICs), however, HW performance is often inadequate. To characterize the effectiveness of strategies to improve HW performance in LMICs, we conducted a systematic review of 15 electronic databases, 29 document inventories of international organizations, and bibliographies of 510 articles. We included studies of any strategy on any health topic in any language, published or not, with an "adequate" design (e.g., trial with comparison group). After screening, data from relevant reports were double-abstracted and entered into a database. Effect sizes were calculated as adjusted risk differences. We screened >105,000 citations, and 489 studies from 83 LMICs met our inclusion criteria. Many strategies have been tested, usually with multiple intervention components. As studies used numerous outcomes that were not always comparable, this analysis focused on outcomes related to processes of care (e.g., % of patients correctly diagnosed or treated). We found that most strategies had small effect sizes (<10 %-points). Effect sizes of strategies that programs often use to improve performance (training alone [n=23 studies], training + job aids [n=19 studies], and supervision alone [n=14 studies]) were modest, typically from 7-10 %-points. Strategies with higher effect sizes (29-47 %-points) included quality management (e.g., team-based problem solving), provision of drugs, community activities (e.g., community health education), and new systems (e.g., new drug management system)--although inclusion of these components did not guarantee high effectiveness. Contextual and methodological heterogeneity made comparisons difficult, and standardization of methods should be a priority of future research. These results, which are based on the largest review of its kind, raise serious concerns about the effectiveness of strategies often implemented by programs and supported by donors in LMICs. The review also identified promising strategies with substantially larger effect sizes.

78

GLOBAL HEALTH SELECTIVE: A NOVEL INTERDISCIPLINARY CLERKSHIP ON CLINICAL KNOWLEDGE AND SKILLS IN GLOBAL HEALTH AT NEW YORK UNIVERSITY SCHOOL OF MEDICINE

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Global health (GH) spans every scientific, clinical and social science discipline. Cultural competency/ cross-cultural sensitivity has been identified as a GH priority for U.S. medical schools (Peluso, 2013). As part of Curriculum for the 21st Century (C21), the Global Health Selective is prerequisite to the new Global Health Concentration at NYU School of Medicine (SoM). With special emphasis on cultural competency/ cross-cultural sensitivity, its primary aim is to teach future physicians fund of knowledge and clinical skills that strengthen GH care. As a 4-week clinical clerkship, the GH Selective was first completed by 9 medical students in 2012, and again by 12 medical students in 2013. Activities included 18 ninety-minute patient case discussions in tropical medicine; related clinical assignments at NYU; literature review and journal clubs; and 9 half-day clinical skills simulation workshops covering 1) diarrhea in Haiti and Egypt, 2) tuberculosis in Peru, 3) malaria in sub-Saharan Africa, 4) hypertension screening by community health workers in Ghana, 5) survivors of torture from central Africa, 6) humanitarian response to tsunami in Indonesia, 7) obstetrical emergencies in rural Liberia, 8) interpreter exercise in Tibetan, and 9) smoking cessation via interpreters. Leadership is from NYU SoM Departments of Medicine and Population Health, and Center for Healthful Behavior Change. Over two years of the GH Selective, student feedback was overwhelmingly positive. Each year, at least 37 faculty volunteered from 11 departments at SoM to log at least 225 hours of direct contact teaching hours each offering. In its first two years, the GH Selective exceeded expectations. Its interdisciplinary curriculum is a particular strength, and its special emphasis on working with standardized patients in cross-cultural settings, focused on communication skills, health literacy, and health navigation, provided students with knowledge and clinical skills applicable for any clinical care provided locally, nationally, and worldwide.

79

THE COST OF DENGUE ILLNESS IN THE PHILIPPINES

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Dengue is a major health problem in the Philippines with an annual average of 118,080 reported cases between 2008 and 2012, placing the country fourth in dengue burden in Southeast Asia. However, the disease surveillance system is designed to predict epidemics, not to report the dengue burden comprehensively. Understanding the real burden of dengue and the cost associated with it can help policy makers evaluate the benefit and effectiveness of new technologies for dengue control and prevention. A four-part study is estimating the direct cost of dengue illness in the Philippines. (1) Expert opinion was gathered through the Delphi method at a national workshop on topics such as the share of cases reported, the proportions of patients treated in hospitals (compared to ambulatory settings), and those treated in the private sector. (2) A desktop study collected, evaluated, and integrated available literature and current knowledge of dengue in the country. (3) Dengue patients' data from the Philippine Health Insurance Corporation (Philhealth) was analyzed to evaluate the charges, payments, length of stay, and distribution of cost between households and Philhealth members. (3) A macro-costing analysis was performed in four tertiary hospitals to estimate the equivalent cost of a hospitalized bed per day, and therefore the direct cost of dengue illness. Adjusting the national surveillance data based on: 1) experts' opinions that 65% of dengue cases were hospitalized, and 47% of these hospitalizations were in the private sector, and 2) the 7.2 expansion factor from a prospective cohort study in Punta Princesa, Cebu city results in an average of 1.13 million annual dengue cases. Of which 833,000 were hospitalized cases. Integrating this information with data collected from public hospitals, Philhealth, and the macro costing analysis yields a $443 million direct cost of dengue illness ($494.67 per hospitalized case and $103.33 per ambulatory case). Of the total cost, households pay 57% from out-of-pocket, governments 20% through operating public facilities, and Philhealth 23% through payments for hospitalized members. If current pilots prove accurate, new dengue control technologies could halve the cases. Such a result would save more than $200 million to the Philippine economy, with the savings benefiting all sectors.

80

CSTOCK - A SIMPLE, AFFORDABLE MHEALTH SOLUTION FOR IMPROVING VISIBILITY OF COMMUNITY HEALTH LOGISTICS DATA

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An assessment conducted in 2010 in Malawi by the SC4CCM Project in collaboration with the Ministry of Health (MOH) showed only 27% of CHWs on the day of the survey had the medicines needed to treat all three targeted childhood illnesses that they were trained to treat - diarrhea, malaria and pneumonia. At the same time, central and district level managers had little access to community logistics data as only 43% of community health workers (CHWs) reported this data to their resupply health center, and little to none of that data reached district or national levels. With 94% of CHWs owning simple mobile phones, the project chose to design and implement an mHealth solution that was simple, affordable, interoperable and could be sustained by the country. The system was designed to provide district and central decision makers regular access to this data to improve decision making and the flow of essential medicines to the community level. cStock, an open-source SMS-based reporting and resupply system with a web-based dashboard, was tested in six districts in Malawi. To promote sustainability, cStock relies on CHWs using their own mobile phones to send messages to a toll free number. CHWs are required to report on two data items and from this over 10 indicators can be monitored. Eighteen months after implementation a mixed-methods evaluation of the intervention was conducted. The evaluation found cStock: 1) improved visibility into stock data (reporting rates now above 80%); 2) was now a primary means for CHWs to order medicines (93% use cStock instead of other forms); 3) saved significant time in CHWs' submitting reports (99% of CHWs); and 4) yielded data that was used by district coordinators for planning and coordinating. cStock has now been scaled up to 15 of the 30 districts in Malawi and has proven to be an affordable and effective way to improve data visibility: an important step in paving the way for supply chain integration. As one CHW said about cStock "the report goes the fastest and gets me the supplies I need in time".

81

COMPARATIVE ANALYSIS OF THE SOCIAL FACTORS THAT INFLUENCE EXPOSURE TO ZOONOTIC DISEASES IN SOUTHEAST ASIA

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Most emerging infectious diseases are zoonotic, with bats, non-human primates or rodents the most frequently implicated sources of disease. Recent increases in emergence have raised concerns about effective

strategies for preventing transmission and mitigating impact. The first step to designing strategies is understanding the context and determinants of disease transmission. The PREVENT project, funded as part of USAID's Emerging Pandemic Threats (EPT) program, is implementing a multi-country series of studies to understand the human-animal interface that can result in risky exposures. We report on results of studies carried out in Thailand, Cambodia and Lao PDR in 2011 and 2012 among different ethnic groups living in varied settings. A premise of the human-animal exposure studies is that disease transmission occurs at the intersection of biological and social processes. While biological factors, such as the number and variety of animal hosts, play a major role, opportunities for transmission depend on social factors that determine human activity: how, where, and when people interact with animals. The human-animal exposure studies examine the effect of social factors, including culture, gender, age, and setting (e.g. urban/rural), on exposure. The current analysis focuses on data from qualitative methods employing participatory tools. Findings indicate varying degrees of exposure to animals depending on location and/or culture. For instance, the acceptability and use of certain animals for food or medicine is a result of culture as well the availability of the animal; while animals such as bats are considered edible by the Lao and the culturally related Isan-Thai, they are not eaten widely in Thailand due to availability and legal restrictions. On the other hand, the Hmong and the Lao living in the same environment have significantly different relationships with animals. In addition, gender and age influence division of labor (e.g. men hunt big animals and women and children capture rats) and restrict involvement in certain activities (e.g. consumption of uncooked meat is discouraged in some of the communities for women). In contrast, other types of exposures, such as rodent infestation, seemed to cross most social boundaries in almost all the settings. This comparative analysis furthers our understanding of the complexity and dynamics of the interplay of factors that result in the emergence of new infectious diseases.

82

REGULATION OF T CELL ACTIVATION IN LEPROSY AND ITS IMMUNE REACTIONS

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There are over 200,000 new cases of leprosy (Hansen's disease) diagnosed each year, 15% of them in Brazil. Leprosy is a spectrum of clinical disease caused by *Mycobacterium leprae,* and ranges from a localized, Th1-predominant tuberculoid (TT) leprosy to a more disseminated, Th2-predominant lepromatous (LL) leprosy. The borderline types (BT, BB, and BL) result from a mixed Th1-Th2 response. Approximately 30% of people with leprosy develop severe immune reactions. Reversal reaction (RR) is an augmentation of the Th1 response to antigen that causes inflammation of skin and nerves in BT, BB, and BL cases. Erythema nodosum leprosum (ENL) is an immune-complex mediated disease in people with BL or LL forms. The goal of this research is to understand the role of T-cell activation in the pathogenesis of leprosy reactions with a hypothesis that people with RR have increased expression of activation markers and lower levels of inhibitory molecules in response to *M. leprae*. Peripheral blood mononuclear cells (PBMC) were isolated from people with leprosy with RR (n=7), ENL (n=7), or without reaction (n=21) and cultured with and without *M. leprae* sonicate. Markers of T cell regulation and cytokine production were assessed using cell surface and intracellular cytokine staining and flow cytometry. The RR group had increase in percentage of CD4+ lymphocytes expressing CD25 and CD69 in response to *M. leprae* ($p<0.05$), and also an increase in CTLA4 in response to antigen. There was a greater percentage of CD4+ lymphocytes producing IFN-γ in culture with or without antigen in RR compared to non-RR ($p<0.05$). The ENL and non-ENL groups had increased CD69 expression in response to *M. leprae* ($p<0.05$), with a trend towards increase in CTLA4. IFN-γ increased in ENL, but not non-ENL, in response to *M. leprae*. The combination of increased expression of markers of T-cell activation and inhibition in response to *M. leprae* in ENL and RR suggests that regulatory mechanisms remain active during the immune cascade of leprosy immune reactions.

83

APPLICATION OF *MYCOBACTERIUM ULCERANS* WHOLE GENOME SEQUENCING TO UNDERSTAND *BURULI* ULCER TRANSMISSION

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Buruli ulcer is a serious disease caused by infection of subcutaneous tissue with *Mycobacterium ulcerans*. Unfortunately disease control efforts are hampered because we do not understand how *M. ulcerans* is transmitted to humans. The clonal nature of *M. ulcerans* has made it difficult to use traditional genetic fingerprinting methods for source tracking and reconstructing transmission pathways. However the advent of cost-effective whole genome sequencing (WGS) has provided a powerful tool to discriminate any *M. ulcerans* isolates. We have begun to apply WGS to *Buruli* ulcer endemic areas in central Ghana and south eastern Australia. Sequencing and comparing the genomes of more than 50 *M. ulcerans* isolates from human and environmental sources in these regions - where the provenance of the isolates is well documented - has highlighted both promise and problems with this approach. Aside from technical issues surrounding optimal analysis of the data, knowing how much genome variation between isolates should be considered significant is critical. Our foray into whole genome sequencing to support *Buruli* ulcer epidemiological investigations is just beginning, but our initial findings indicate significantly more isolate variation even at the village scale than we previously anticipated, encouraging us to consider the existence of multiple environmental reservoirs of *M. ulcerans*. As we determine more WGS from carefully selected isolates with well-documented histories, the utility of this approach will greatly improve and lead us to a deeper understanding of *Buruli* ulcer transmission.

84

MICRO-EPIDEMIOLOGICAL APPROACH TO UNDERSTANDING TRANSMISSION DYNAMICS OF *MYCOBACTERIUM ULCERANS* IN THE OUÉMÉ RIVER VALLEY IN SOUTHERN BENIN

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Buruli ulcer (BU) is a necrotizing skin and bone disease caused by the enigmatic pathogen *Mycobacterium ulcerans* in riverine regions of West and Central Africa. The highly clonal nature of *M. ulcerans* has complicated molecular analyses on the epidemiology of the pathogen, as typing methods with sufficient resolution have been lacking. Increased availability of Next Generation Sequencing techniques now allow us to address previously unanswerable questions, such as how BU is transmitted to humans. The addition of classical epidemiology at the village level to the

bacterial genomic data, will provide a novel approach to unraveling the enigmatic nature of *M. ulcerans* transmission. In the commune of Ouinhi in the Ouémé river valley in southern Benin, all BU patients are clinically, demographically, geographically and microbiologically well-documented. We use different approaches to study the possible role of a human reservoir in BU transmission in this region. We are retrospectively analyzing spatiotemporal clustering of BU patients and *M. ulcerans* genotypes on isolates collected between 1989 and 2011. We will report results on 239 *M. ulcerans* cultures isolated between 2000 and 2010 from different patients living in the study area. The genomic DNA of the *M. ulcerans* isolates was sequenced using an Illumina MiSeq sequencer. A Python utility called Nesoni was used to map sequence reads to the Ghanaian Agy99 *M. ulcerans* reference genome and to identify variant sites. These were concatenated to form a multiple alignment, from which a phylogenetic tree was constructed using SplitsTree4. Preliminary results on a first set of 19 bacterial genomes from this focused geographic region reveal 11 to 54 SNP differences. More whole genome sequences are required to give context to the spatiotemporal variation seen. We expect that this study, unparalleled in size, duration and comprehensiveness, will provide fundamental insight in the transmission dynamics of *M. ulcerans*. Such understanding is central to the development of strategies to track and interrupt the spread of the disease.

85

SPATIAL ANALYSIS OF HUMAN ACTIVITY, *BURULI* ULCER AND *MYCOBACTERIUM ULCERANS* ALONG THE COUFFO RIVER DRAINAGE IN BENIN

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Buruli ulcer is a severe cutaneous infection that is widespread in West and Central Africa. The causative agent is *Mycobacterium ulcerans,* an environmental pathogen. Transmission occurs through contact with an infected environment; person-to-person transmission is extremely rare. The mode of transmission of *M. ulcerans* from the environment to humans remains a central mystery of *M.* ulcerans research. We have conducted detailed, small-scale studies on the distribution of *M.* ulcerans in the environment, gathered basic demographic data, and mapped cases to individual residences in 10 villages/hamlets in Lalo Commune. Results from these studies reveal considerable variation at local spatial scale between communities in village/hamlet size, extent of out-migration versus year round residence, agricultural/livelihood activities, ethnicity, age structure and spatial patterns of *Buruli* ulcer. Studies on the distribution of *M. ulcerans* in the environment based on results from quantitative PCR show an extremely heterogeneous distribution. Whereas the concentration *M. ulcerans* in environmental samples taken within the village center is low, much higher concentrations of *M. ulcerans* are detected in agricultural/ livelihood spaces peripheral to the village. Over 97% of positive samples are from aquatic sources including water filtrand, aquatic invertebrates and macrophytes. *M. ulcerans* is rare in "dry" agricultural sites such as maize fields and oil palm groves, but is present in high concentrations raffia palm swamps and rice fields. These findings suggest that "location" is a far more important determinant of infection than specific activity, such as "agriculture". The identification of "high activity spaces" where human activities intersect with habitats highly contaminated with *M. ulcerans* provides key information for further focused studies on transmission.

86

EARLY INFECTION OF *MYCOBACTERIUM ULCERANS* IN A GUINEA PIG MODEL

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The transmission of *Mycobacterium ulcerans* from the environment to humans remains an enigma. Hypotheses include that *M. ulcerans* is acquired through an insect vector or that bacteria enter open wounds through exposure to a contaminated environment. We have previously reported development of an animal model to study these hypotheses. From this, we were able to produce an infection 100% of the time when the inoculum was injected intradermally, but were unable to establish an infection through application of *M. ulcerans* to an open abrasion. Here, we report from a 14-day study to examine early timepoints, and have also included *Staphylococcus aureus* as a positive control for inflammation and topical infection. Hairless Hartley guinea pigs were infected with *M. ulcerans* by injection or by application to an open abrasion, and *S. aureus* was applied to abrasion sites of two guinea pigs as a positive control. We compared the efficiency of transmission routes by qPCR, histopathology, and culture. Abrasions healed within 7 days. *M. ulcerans* was isolated from abrasion sites up to 24 hours post infection, but later time points were uniformly negative. *M. ulcerans* genome units were detected from abrasion sites at every time-point except at the final, 14 day time-point. In contrast, lesions were apparent at the injection site within 7 days, all injection sites were positive when assayed via qPCR with high copy numbers of genome units, and *M. ulcerans* were recovered from all injection sites upon culture. Microscopic evaluation of abrasion sites infected with *S. aureus* revealed extensive colonization of the upper dermis where huge numbers of gram positive cocci could be seen decorating cells and the lamnia propia. Gross pathology showed extensive vascularization and other signs of inflammation. Acid-fast bacteria were rarely detected in abrasion sites and occurred as a large cluster of organisms that did not appear to be cell associated. These results suggest that *M. ulcerans* can only establish infection through injection and has implications toward transmission.

87

ASSESSMENT OF BACTERIAL BURDEN OF *BURULI* ULCER (BU) LESIONS: A CALL FOR CLEAR GUIDELINES ON WOUND CARE MODULE FOR BU CASE MANAGEMENT

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Buruli ulcer (BU), caused by *Mycobacterium ulcerans* (MU) is a chronic necrotizing skin disease. BU typically starts as a subcutaneous nodule or plaque containing large clusters of extracellular acid-fast bacilli. Extensive necrosis by the cytotoxic macrolide toxin, mycolactone produced by MU leads to destruction of subcutaneous fat and soft tissue of the affected skin leading to the formation of large ulcers that progress, if untreated. In a cross-sectional survey we analyzed the bacterial flora of BU lesions of three different groups of patients before, during and after daily treatment with streptomycin and rifampicin for eight weeks (SR8) and determined drug resistance of the bacteria isolated from the lesions. Subsequently we also followed a new cohort of cases prospectively during BU case management. Within the cross-sectional survey, we found more than 60% of analysed wounds infected by other bacteria before SR8 treatment,

with *Staphylococcus aureus* and *Pseudomonas aeruginosa* being the most prominent ones. During treatment 65% of all lesions were still infected, mainly with *P. aeruginosa*. After completion of SR8 treatment still more than 75% of lesions clinically suspected to be infected were microbiologically confirmed as infected, mainly by *P. aeruginosa* or *Proteus miriabilis*. Infections were also confirmed by histopathology. Twelve cases were then followed prospectively and of these, we found that the microbiological burden of wounds before and during treatment had median values of 2.4 x106 and 7.8 x 105 respectively. The burden then increased significantly post SR8 ($p<0.01$) to a median and mean values of 1.6 x109 and 2.7 x109 respectively. Drug susceptibility tests revealed especially for *S. aureus* a high frequency of resistance to the first line drugs used in Ghana. Our results show that secondary infection of BU lesions is common and the significant increase in bacteria burden after antibiotic treatment calls for proper wound care module for BU case management.

88

LONG TERM STREPTOMYCIN TOXICITY IN THE TREATMENT OF *BURULI* ULCER: FOLLOW-UP OF PARTICIPANTS IN THE BURULICO DRUG TRIAL

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Buruli Ulcer (BU) is a Neglected Tropical Disease occurring predominantly in West-Africa. Symptomatic infection typically causes a nodular skin lesion which eventually ulcerates. If the ulcer is left untreated, functional limitations can result due to scarring and contractures. The current WHO-recommended treatment is 8 weeks of intramuscular streptomycin and oral rifampicin, which is successful in healing the disease in early stages. However, side-effects are a concern, as prolonged streptomycin administration can cause both oto- and nephrotoxicity. We therefore evaluated its long term toxicity by retrieving former BU patients that had received either 4 or 8 weeks of streptomycin in addition to other drugs between 2006 and 2008, in the context of a randomized controlled trial. Former patients were retrieved in 2012, and oto- and nephrotoxicity were determined by audiometry and serum creatinine levels. Data were compared with baseline and 2, 4, 6, and 8 week measurements during the drug trial. Of the total of 151 former patients, 127 (84%) were retrieved. Ototoxicity was present in 29% of adults and 25% of children. Adults in the 8 week streptomycin group had significantly lower hearing thresholds in all frequencies at long term follow-up, and these differences were most prominent in the high frequencies. In children, no differences between the two treatment arms were found. Nephrotoxicity that had been detected in 14% of adults and in 13% of children during treatment, was present in only 2.4% of patients at long term follow-up. Prolonged streptomycin administration in the adult study subjects caused significant permanent hearing loss, especially in the high frequency range; importantly, this hearing loss was not self-reported on questioning. Nephrotoxicity was also present in both adults and children but appeared to be transient. We conclude that streptomycin with cumulative dosages, especially in patients aged 16 or older should be given with caution, especially in individuals with concurrent risks for renal dysfunction or hearing loss.

89

WESTERN VERSUS EASTERN AFRICAN EXPERIMENTAL HUTS FOR THE EVALUATION OF PRODUCTS: A SWOT ANALYSIS FROM COMPARATIVE TEST OF REPELLENTS AND INSECTICIDAL PRODUCTS IN BENIN

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The Western and Eastern African experimental huts are used to assess the efficacy of products targeting mosquitoes. The range is vast and includes toxicants and spatial repellents that keep mosquitoes away from human. Owing to differing design of the west versus east type, the suitability of either hut to evaluate a given product is questionable. The present study compared the efficacy of Spatial Repellents (coils) versus Long Lasting Insecticidal Net in both design and highlighted their Strength and Weaknesses. Olyset Net and metofluthrin 0.00625% 0.0097% were evaluated in Southern Benin. The Western huts have mosquitoes entry slits on the sides and large screened verandah to prevent egress of mosquitoes. The Eastern designs have entry baffles and eave gaps surrounding the roof through which mosquitoes escape or access huts. Only *Culex quinquefasciatus* collected in abundance was analysed further and reported. Both type of huts reduced entry of *Culex* into huts in presence of the coils or LLIN but the rate of entry was reduced by 78-79% in the western design compared to only 28-49% in the eastern format ($P<0.05$), with Olyset deterring the least. Without treatments, the proportions of mosquitoes exiting the Eastern huts by dawn (64%) were greater than those caught in veranda of the western huts (34%) and this was still evident with the treatments ($P<0.05$). The overall personal protection levels were similar in the eastern and western huts for the Olyset Net (94-95%) but significantly higher in the eastern hut than the western hut for the spatial repellents (49-62% vs 39-52%) ($P<0.05$). Induced mortality was lower in the western hut for all treatments compared to the eastern design. The study showed the suitability of both type of huts to evaluate key properties (blood feeding inhibition and toxicity) induced by non-spatial repellent or lowly deterrent intervention like olyset Net though a slight but significant improvement was observed with the Ifakara experimental hut. Spatial repellents are better suited to the eastern experimental huts to deliver protection through higher exophily than the confined western design, which current structure is not suited to assess accurately deterrence and exophily induced by chemicals.

90

DIVERSITY OF SAND FLY SPECIES IN THE HO-KPANDO DISTRICT OF GHANA

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In 1999, cases of Cutaneous Leishmaniasis (CL) were diagnosed in the Ho district of the Volta Region of Ghana, although the causative parasite, the reservoir host and the sand fly vector for the disease were not clearly determined. Previous studies in the Ho region have suggested *Sergentomyia ingrami* and *S. hamoni* as possible vectors of CL in Ghana. In 2008, samples of indoor collected sandflies from communities close to Kpandu in the Volta Region, known to be endemic for CL showed the presence of different species belonging to the genus *Sergentomyia*. No *Phlebotomus* species were identified. A total of 686 sandflies belonging to 4 subgenera namely, *Parrotomyia, Grassommyia, Neophlebotomus* and *Sergentomyia* were identified by morphological characteristics and polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP). The major sandfly species were *S. africana africana* (30%), *S.*

simillima (26.2%), *S. ghesquierei* (20.6%), *S. ingrami* (18.8%), *S. collati* (1.1%), *S. dureni* (1%), *S. hamon*i (0.4%), *S. antennata* (0.4%), *S. inermis* (0.3%), *S. buxtoni* (0.3%), *S. schwetzi* (0.3%) and *S. squamipleuris* (0.3%). Earlier collections in CL endemic areas in a more southern part of the Volta Region in 2006-2007 showed a majority of S. africana africana as well, although these were found at rates of 80% and above. Although these results are inconclusive, they suggest the need for a wider surveillance in areas endemic to leishmaniasis. *Sergentomyia* species are not known as vectors for leishmaniasis, however, there is an increasing suspicion that it may be a potential vector of CL, occurring in the Volta Region.

91

ANTI-CHYMOTRYPSIN ACTIVITY OF TWO SERPIN MOLECULES FROM THE TICK *RHIPICEPHALUS HAEMAPHYSALOIDES*

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Two novel serpins with anti-chymotrypsin activity, RHS-1 and RHS-2, were identified in the tick *Rhipicephalus haemaphysaloides*. The complementary (c)DNA sequence of RHS-1 was 1286 base pairs (bp) and encoded a deduced 403-amino acid protein with a signal peptide, whereas that of RHS-2 was 1682 bp and encoded a deduced 380-amino acid protein with no signal peptide. Although both RHS-1 and RHS-2 exhibited high sequence similarities to known serpins from other ticks, the level of similarity at the amino acid level between the two serpins characterized here was only 32.5%. Salivary gland-specific expression of RHS-1 and midgut-specific expression of RHS-2 were found by Western blot using the relevant antiserum. We tested the ability of purified recombinant (r) RHS-1 and rRHS-2 to inhibit various serine proteases and found that both significantly inhibited chymotrypsin (95.6% and 94.2%, respectively). We further demonstrated that RHS-1 but not RHS-2 exhibited anticoagulation activity, based on activated partial thromboplastin time (APTT). Disruption of the genes encoding the two serpins with RNA interference (RNAi) led to a significant decrease in tick attachment and engorgement rates. These results indicate that RHS-1 and RHS-2 are two novel serpins with anti-chymotrypsin activity that are involved in blood feeding by *R. haemaphysaloides.*

92

THE SALIVARY SECRETOME OF THE BITING MIDGE, *CULICOIDES SONORENSIS*

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Culicoides biting midges (*Diptera: Ceratopogonidae*) are hematophagous insects with over 1400 species distributed throughout the world. Many of these species are of particular agricultural importance as primary vectors of bluetongue, epizootic hemorrhagic disease, Schmallenberg, and African horse sickness viruses. Detailed studies of members from other blood-feeding Diptera, including mosquito (Culicidae) and black fly (Simuliidae), have shown that protein components within saliva are critical in the blood feeding process. To determine the protein components in *Culicoides sonorensis* midges, the primary vector of bluetongue virus in the U.S., secreted saliva was collected and analyzed by tandem mass spectrometry peptide sequencing. Fifty-two secreted proteins were identified, including members of the D7 odorant binding protein family, Kunitz-like serine protease inhibitors, maltase, trypsin, and six novel proteins unique to C. sonorensis. Possible roles of identified salivary proteins in facilitating blood feeding, as well as arbovirus transmission and pathogenesis are discussed.

93

EFFECTIVE CONTROL OF *AEDES AEGYPTI* USING CDC AUTOCIDAL GRAVID OVITRAPS

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We have previously shown that using 3-4 sticky, CDC Autocidal Gravid Ovitraps (AGO trap) per home reduced the *Aegypti aegypti* population in 60% and prevented the development of mosquito outbreaks in southern Puerto Rico, where the impact of the AGO traps was compared in two isolated urban areas (with or without AGO traps) for one year. After demonstrating treatment effectiveness, we deployed 3 AGO traps per home in both the area that formerly served as the reference site (without AGO traps) and the site that served as the intervention area. Two nearby urban areas were selected as the new reference areas to compare the density of *Ae. aegypti* without and with AGO traps. We monitored mosquito density using sentinel AGO traps every week in all four sites and placed meteorological stations to record rainfall and temperature. The hypotheses tested were: the density of *Ae. aegypti* in the former reference area converges to the low levels observed in the intervention area and mosquito density in both areas having AGO control traps is significantly lower than in the new reference areas. Preliminary results provide strong support for both hypotheses. Mosquito density in the former reference area has dropped even below that observed in the former intervention area and mosquito density in the new reference areas has been several times greater than in the intervention areas. These results seem to confirm that AGO traps are effective control tools for *Ae. aegypti*.

94

ASSESSMENT OF LARVAL THERAPY FROM *SARCONESIOPSIS MAGELLANICA* (*DIPTERA: CALLIPHORIDAE*) IN AN ANIMAL BIOMODEL

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Larval therapy is a safe and economical method to promote the healing of infected and necrotic wounds. The aim of this study was to evaluate the larval therapy using *Sarconesiopsis magellanica* in an animal model. This species of the Calliphoridae family is of medical and forensic importance. Twelve rabbits were used in experiments, bearing previous approbation of the Ethics Committee of the Rosario University. A wound was carried out in each rabbit and afterwards 1 mL of bacterial suspension of both *Staphylococcus aureus* and *Pseudomonas aeruginosa* were inoculated into wounds. The animals were divided into four groups: the first was treated with larval therapy from *S. magellanica*, the second with *Lucilia sericata* larvae, the third was treated with antibiotic and the last group was established as a negative control. The healing process was evaluated through both macroscopical and histopathological methods. The assessment from rabbit's wounds treated with larval therapy showed bacterial reduction and removal of necrotic tissues. The wound healing occurred approximately 25 days post-treatment, but the granulation tissue appeared earlier in rabbits treated with larval therapy, which had less exudates compared with controls. Histological evaluation evidenced inflammation in all treatments on day six. However, groups treated with larval therapy reached the cell proliferation stage in less time. This research demonstrates the efficacy of *S. magellanica* as a new alternative to use in larval therapy.

95

PROTEOLYTIC ACTIVITY OF *SARCONESIOPSIS MAGELLANICA* (*DIPTERA: CALLIPHORIDAE*) LARVAL EXCRETIONS/ SECRETIONS

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Sarconesiopsis magellanica is a necrophagous blowfly having medical and forensic relevance, which produces facultative myiasis in some mammals and is frequently used to establish post-mortem interval. Proteases contained in the larval excretions/secretions (E/S) contribute in the healing process of necrotic wounds during larval therapy. This study aimed at identifying and characterizing for the first time the proteolytic enzymes present in E/S of *S. magellanica* in third instar larvae taken from an established colony. E/S proteins were size-separated by SDS-PAGE and their proteolytic activity was assessed in zymograms; in addition, inhibition assays using BAPNA and SAPNA as substrates, and synthetic inhibitors were carried out. The protein profile showed bands ranging from 80 to 10 kDa, displaying those from 50 to 10 kDa a similar pattern to that seen in *Lucilia sericata* with a predominant 25 kDa band. Zymography showed the highest proteolysis at 42 and 25 kDa. Three protease types were found: serine-, cysteine- and metallo-proteases. Reduced proteolysis was observed when PMSF and TLCK inhibitors were added at pH 7.5, when BAPNA was used as substrate, suggesting the presence of trypsin-like serine proteases; the proteolysis of SAPNA was significantly inhibited when the chymotrypsin-specific TPCK was used as inhibitor at the same pH. These data suggest that proteases present in E/S of *S. magellanica* might help in the healing process of necrotic wounds and be potentially useful in larval therapy.

96

BIOLOGICAL EFFECTS OF *SARCONESIOPSIS MAGELLANICA* (*DIPTERA: CALLIPHORIDAE*) LARVAL EXCRETIONS/ SECRETIONS ON FIBROBLASTS

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Larval therapy promotes debridement, disinfection and stimulates granulation tissue in chronic wounds. Larval excretions/secretions (E/S) play an important role in controlling fibroblast proliferation, adhesion and migration. *Sarconesiopsis magellanica* is a necrophagous and hemisynanthropic blowfly having medical and forensic relevance. The *in vitro* effect of E/S on fibroblast cell cultures was here assessed. E/S were obtained from third instar larvae from an established colony. Viability and cell proliferation were evaluated in an MTT assay; morphology, adhesion and cell migration were also assessed in multiwell plates covered with collagen and follow up was carried out by microscopy. Parallel assays with E/S heated at 90ºC for 1h were done. E/S from *S. magellanica* increased fibroblast viability and proliferation at concentrations of 5 and 2.5 µg/mL, in addition, diminished cell adhesion with increased migration was observed. E/S induced a reduction in cell surface accompanied with morphological changes. No significant differences were observed when fibroblasts were incubated with pre-heated E/S, suggesting that the changes observed previously might have been due to protease activity. These data suggest that E/S from *S. magellanica* could promote fibroblast migration in chronic wounds, facilitating tissue regeneration.

97

THE ANOPHELINE ANTI-FUNGAL DEFENSE SYSTEM

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Anopheline mosquitoes rely primarily on their Toll and IMD innate immune systems to defend against pathogen infection. Although the Toll pathway is implicated in anti-fungal defense, the role of the IMD pathway remains poorly understood. We used field-collected fungi from Puerto Rico to probe interactions with the mosquito immune system. Ingestion of two species of filamentous fungi resulted in transient activation of the mosquito's IMD pathway, suggesting this pathway could be involved in anti-fungal defense. We observed that Rel2 transgenic *A. stephensi* mosquitoes survived significantly longer than wild type controls when infected with the entomopathogenic fungus *Beauveria bassiana*, further implicating the IMD pathway in anti-fungal defense. Interestingly, mosquitoes that ingested filamentous fungi became more susceptible to *Plasmodium* infection. These results have implications for our understanding of *Anopheline* innate immunity. We are further investigating if exposure to fungi could account for disparities in *Plasmodium* susceptibility in nature.

98

HOUSE AND HUMAN INFESTATION BY THE TROPICAL RAT MITE *ORNITHONYSSUS BACOTI*, A CASE REPORT IN LIMA, PERU

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Mite bites from animals is one of the cause of human dermatitis. One of those parasites is the Tropical rat mites (*Ornithonyssus bacoti*), a common ectoparasite in a variety of small rodents such as rats, mice, hamsters, gerbils, voles, and other wild rodents. This work reports a case of human dermatitis and house infestation by *O. bacoti* in Lima, Peru. A 5-member-family (father, mother and three children) had papule from 1 to 4 mm in diameter in arms, back and thighs. Likewise, they presented dermatitis and pruritus on the affected zone. The family lives in a second-floor apartment in an urban area of Lima. After 6 days of starting the problem, the mother found tiny brown and red bugs on the bed of the children and parents, principally between the bed sheets. The bugs were collected and taken to the Laboratory of Veterinary Preventive Medicine, School of Veterinary Medicine, San Marcos University for diagnosis. Morphological diagnostic concluded that the species correspond to the Tropical rat mite *O. bacoti.* We went to visit the house to see if there was problem with rodents (mouse and rats). However, there was not a rodent problem; instead the bedrooms of the parents and children were highly infested with *O. bacoti*. We observed mites even in the edge of the window and corners of the bedrooms. The father told us that the children had two hamsters as pets, and they died 2 weeks before the onset of the dermatitis problem. The hamsters had lived in a plastic cage in the children's room. We suspect that the hamsters were infected with the mite and after they died the parasite spread in the room. Many reports have shown that rodent pets such as hamsters and gerbils are source of direct transmission of *O. bacoti* to humans. It is necessary to evaluate and inform the zoonotic importance of this parasite in Veterinary hospitals and Clinics, as well as pet shops.

99

ENTOMOLOGICAL EVALUATION OF *CULICOIDES* SPECIES AND THEIR POTENTIAL ROLE IN *MANSONELLA PERSTANS* TRANSMISSION MALI

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Mansonella perstans (Mp) is a filarial parasite of humans that is highly endemic in central and west Africa, including Mali, with a prevalence of infection approaching 100% in some regions. Since the majority of infected individuals are asymptomatic, Mp has been largely neglected, and little is known about the entomology of the vectors that transmit infection in West Africa. To begin to address this issue, we conducted a longitudinal and cross sectional study of *Culicoïdes* species in two Malian villages known to be highly endemic for Mp, Boundioba (Kolondieba district) and Tiénéguébougou (Kolokani district). Vectors were collected outdoors and inside houses using light traps from January to December 2012, in order to (1) perform an inventory of *Culicoïdes* species and (2) investigate the involvement of the *Culicoïdes* genus in Mp transmission. Random sampling was used for species inventory, and systematic sampling was used for the evaluation of Mp entomological parameters. Preliminary results from the first 3 months (January to March 2012) identified 9 species of *Culicoïdes* in Kolokani and 4 in Boundioba. The *Culicoides* genus comprised 20.34% (n = 12,867) and 1.43% (n = 7196) of all insects collected in Boundioba and Tiénéguébougou, respectively. Although the total number of vectors captured increased progressively from January to March in both villages, the percentage of *Culicoides* among the vectors captured outdoors also increased (from 6% to 32% in Boudioba and from 1% to 4% in Tiénéguébougou). *Culicoides* prevalence did not increase over time among vectors captured indoors. To assess for the presence of *M. perstans*, *Culicoides* vectors were divided into pools and DNA was extracted for PCR analysis using Poolscreen. Among 704 vectors tested to date, no positives have been identified. Although these preliminary data do not demonstrate a link between *Culicoides* species and Mp transmission in Mali, the number of vectors assessed to date is insufficient to exclude this possibility, and analysis of the remaining vectors is underway.

100

PROTEOMIC ANALYSIS OF *SARCOPTES SCABIEI*

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The ectoparasitic mite *Sarcoptes scabiei* burrows in the skin causing intense itching. The disease is difficult to detect since no reliable diagnostic test is currently available. The goal of this research is to characterize the proteome of scabies mites and to identify proteins that may be useful for inclusion in a diagnostic test or in a vaccine to prevent this disease. Washed scabies mites were homogenized in water and the soluble extract was fractionated by 2-dimensional electrophoresis. Proteins were identified by Coomassie blue staining, excised and subjected to in-gel trypsin digestion. Tryptic peptides were analyzed by mass spectrometry and resulting sequences were searched against the SwissProt database. Of the ~200 spots visible on the gels, >100 have been subjected to analysis. The most abundant protein in scabies mite extract was identified as tropomyosin. Parallel immunoblots probed with serum from scabies-infested hosts (humans, rabbits and dogs) did not show antibody binding to this protein. In addition to some host proteins presumably extracted from the mite gut, peptides with homology to several heat shock proteins, actin, arginine kinase and enolase were also identified. Immunoblot analysis revealed that some of these proteins bound antibody in the serum of the scabies-infested hosts indicating that these proteins warrant further study as possibly useful diagnostics.

101

MOLECULAR CHARACTERIZATION OF INSECTICIDE RESISTANCE IN *PHLEBOTOMUS PAPATASI* AND *LUTZOMYIA LONGIPALPIS* SAND FLIES

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Insecticides are a practical pest management tool to reduce bites from *Phlebotomus papatasi* and *Lutzomyia longipalpis*, the primary sand fly vectors of Leishmaniasis, a neglected disease affecting 20 million people yearly. Recently, there have been reports of sand fly insecticide resistance worldwide. Protein target-site insensitivity via single nucleotide polymorphisms (SNPs) specific to pyrethroid and organophosphate insecticides has been documented in insects in the paralytic (para) and the acetylcholinesterase-1 (ace-1) genes. Limited molecular characterization of resistance potential has been performed in sand fly vectors. We hypothesized that *P. papatasi* and *L. longipalpis* colonies resistant to pyrethroids and organophosphates would develop homologous insensitivity SNPs in these genes. Para and ace-1 gene fragments were sequenced in insecticide-susceptible *P. papatasi* and *L. longipalpis*. Survival curves were developed, using 1-hour bottle assays, for *P. papatasi* to determine lethal concentrations of permethrin or malathion insecticides. Approximately 500 *P. papatasi* were exposed to the LC50 of permethrin and another subset to the LC75 of malathion. Flies that survived were blood feed and eggs were collected. Subsequent generations from the *P. papatasi* permethrin and malathion selected colonies were exposed to the same initial insecticide concentration before blood feeding. Survival from the initial insecticide exposure to the F3 generation went from 14.7% to 94.8% for permethrin and from 14.6% to 64% for malathion. Para and ace-1 gene sequences are currently being generated from the F3 selected colonies and undergoing SNP analysis. Initial data suggest there is no insensitivity SNPs that have developed in the para gene sequence. Artificially resistant *P. papatasi* colonies will continue to be exposed to insecticide and will undergo molecular characterization and biochemical assays at the F5 generation. Artificial selection of *L. longipalpis* for permethrin and malathion resistance will be performed once survival curves are complete.

102

ANALYSIS OF KNOWLEDGE AND PERCEPTIONS OF TRIATOMINES AND CHAGAS DISEASE THROUGH CHILDREN'S DRAWING IN FOUR RURAL VILLAGES OF YUCATÁN, MEXICO

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Chagas disease is an underreported neglected tropical disease in Mexico, where no formal Chagas control or prevention programs exist. The southern states of Chiapas, Oaxaca, Puebla, Veracruz and Yucatan are among the highly endemic areas. Very few intervention or education activities exist in these endemic regions, resulting in uncertain knowledge of Chagas disease. The aim of this study was to evaluate children's knowledge, understanding and misconceptions about the insect vector and Chagas disease through a drawing exercise called "The Pic and My House." This study was conducted in four rural villages of Bokoba, Sanahcat, Sudzal and Teya in the Yucatan Peninsula where previous Chagas vector control and education activities have been carried over

the past 8 years. Previous efforts had few activities targeting children specifically, therefore the second aim of this study was to examine if and how previous Chagas activities in these communities have informed children about Chagas. A total of 261 drawings were collected from primary school children, ages 6-12. The images and the messages on the drawings were scored qualitatively using a scoring tool that included 6 thematic categories and 61 subcategories that were inductively and deductively defined. We compared the frequencies of scored themes by village, age and gender. Significant differences were found between villages in relation to location, appearance and behavior of the triatomine, types of hosts, and messages written on the drawings. Despite good overall knowledge displayed in the drawings, certain misconceptions and misunderstandings were identified. The findings show that previous education efforts such as informational pamphlet distribution have made an impact on children's knowledge of Chagas. The results of this study demonstrate that drawings can provide valuable insight into children's knowledge and these findings should be taken into account when designing future education programs specifically targeting children.

103

DENGUE AND CHIKUNGUNYA SEROPREVALENCE IN RURAL COASTAL KENYA

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Dengue virus (DENV) and chikungunya virus (CHIKV) infections cause incapacitating fever syndromes world-wide, yet they are often overlooked by public health and research programs, particularly in Africa. This study was ubdertaken to measure the seroprevalence of DENV and CHIKV in three Kenyan villages, and link seropositivity to demographics and other exposures. Demographic, household inventory and exposure questionnaires were administered to participants in a household-based cluster study of Milalani, Nganja, and Vuga villages in 2009. Sera were tested for exposure to DENV and CHIKV using standardized ELISA protocols. Bivariate relationships for each potential predictor of DENV and CHIKV seropositivity were assessed using a $\chi 2$ test. Multivariable logistic regression was used to further test predictor variables for association with seropositivity. Of the 1862 study participants (560 Milalani, 452 Nganja, and 850 Vuga). Of the remaining 1817 DENV participants, 932 (51%) were DENV seropositive, aged 1 - 99 years. Of the remaining 1818 CHIKV participants, 392 (22%) were CHIKV seropositive, aged 2 - 76 years. 795 (45%) of the samples were from children (≤15 years) and of these, 168 (21%) were DENV seropositive while 144 (18%) were CHIKV seropositive. Of the 978 adults, 761(78%) tested positive for DENV; 246 (25%) tested positive for CHIKV. Children were less likely to be seropositive ($p<0.01$). Women were more likely to be seropositive ($p<0.05$). Seropositives were less likely to own a motor vehicle ($p<0.001$). CHIKV and DENV seropositivity were closely associated ($p<0.01$). Confirmatory plaque reduction neutralization testing is ongoing. In conclusion, dengue and chikungunya are common in rural coastal Kenya. Ongoing interepidemic transmission of CHIKV is demonstrated by many CHIKV seropositive children aged 2 -4, given that the last known outbreak in this area occurred in 2004. Ongoing inter-epidemic transmission of DENV is also supported. Children and those with higher socioeconomic status were less likely to be seropositive. Those exposed to DENV were more likely to be CHIKV seropositive, and vice versa, which is likely a result of the common vectors. Cross-reactivity with other related viruses may have led to false positive results, but it is clear that flavivirus and alphavirus exposure are widespread in this area of Kenya.

104

MODELLING THE GLOBAL EFFECTS OF TEMPERATURE ON SEASONAL VARIATION IN DENGUE TRANSMISSION

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The transmission of dengue virus, like many other vector borne diseases, is dependent on the completion of a number of temperature dependent life cycle stages within the vector and host. While the temperature within the host remains relatively constant, the vector experiences a much wider range temperatures over the course of a single day, season or year that will affect viral replication. To complete the transmission cycle, the adult vector must survive long enough for the virus to migrate, mature and replicate. Both adult vector survivorship and the extrinsic incubation period (EIP) of the virus are temperature-dependent processes. While low and high temperatures have been shown in the field to limit dengue transmission through these two processes, it is unclear what limits temperature exerts on the extent and seasonal variation of transmission at a global scale. Here we show that modelling the temperature-dependent interaction between mosquito mortality and dengue virus EIP explains a significant proportion of spatial and seasonal variation in reported dengue cases. In a meta-analysis of controlled laboratory condition data, we used a Bayesian framework to define two separate temperature relationships between *Aedes aegypti* longevity and virus EIP with associated credible intervals. The two relationships were then combined in an analytical framework and applied to long-term average weekly temperature data at 1km x 1km resolution to produce a global animated map. When combined with reported dengue case data, we show that our index of temperature suitability can reveal seasonal variations in intensity and extent of transmission unaccounted for by simpler temperature correlations. Our results demonstrate that by modelling two important biological processes, significant intra-annual changes in the geographic distribution of dengue suitability can be mapped. The resulting maps can be used to guide surveillance and control resources by site and season and the models can be combined with other environmental data to develop early warning systems for dengue outbreaks.

105

ANTIVIRAL EFFECT OF THE CELLULAR PROMYELOCYTIC LEUKEMIA PROTEIN AGAINST DENGUE VIRUS

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Interferons (IFN) are a family of proteins involved in many cellular processes including antiviral defense. This activity is mediated by well characterized IFN-induced proteins (e.g. PKR kinase, Mx GTPases). Other IFN-induced proteins have been proposed to show antiviral activity including Promyelocytic leukemia protein (PML). PML nuclear bodies (PML-NBs) are discrete nuclear foci that require PML for their formation and contain many different cellular proteins involved in diverse processes such as transcription, apoptosis and antiviral response. PML-NBs have a punctuate appearance when visualized by fluorescence microscopy and their number range between 1 and 30 per cell nucleus. Many viruses express proteins that disrupt PML-NBs. Since PML expression is induced by IFN, disruption of PML-NBs may be a viral strategy to evade the IFN-mediated innate immune response. Here, we investigated the role of

PML in the replication of dengue virus (DENV). To evaluate the antiviral role of PML in DENV multiplication, PML expression was silenced in A549 cells by transfection with PML-specific siRNAs. The virus yield observed in PML-silenced A549 cells was 10-fold higher in comparison to the one obtained in A549 control-siRNAs transfected cells. To further investigate the PML antiviral role, A549 cells were transfected with a PML encoding plasmid and infected with DENV. It was observed that PML overexpression induced partial resistance to DENV multiplication, as 50% inhibition on virus yield was obtained. Confocal microscopy images showed that nuclear PML underwent a dramatic rearrangement during DENV infection. Moreover, in A549 cells not expressing viral antigens, PML-NBs became more distinct, with increased numbers and size. Due to the changes observed by microscopy in PML distribution during viral infection, the PML-mRNA levels were determined by qRT-PCR. A 1000-fold increase in PML-mRNA expression was found in DENV infected A549 cells, relative to mock infected A549 cells. This is the first brief report of the susceptibility of DENV to PML. The exact mechanism behind PML-mediated intracellular defense against DENV requires further study.

106

UNDERSTANDING DENGUE TRANSMISSION IN DHAKA, BANGLADESH

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Developing an appropriate multi-disciplinary research design to apply a "Ecohealth Approach" to understand dengue virus (DENV) transmission in Dhaka, Bangladesh, where the 16 million occupants have been exposed to a resurgence of dengue since 2000. To develop a suitable research design, we considered variation in: socio-economic status among the city-zones, gender inequality, population density, housing, and water supply, waste disposal and sewage systems. Multiple disciplinary aspects were encapsulated by examination of: i) rates of human exposure to dengue virus (DENV) by identifying individuals (via a serosurvey in 1200 households) with IgM and IgG antibodies to DENV and acute cases of illness from hospitals (47 diagnostic study of suspected hospitalized patients) by identifying the presence of DENV RNA by PCR amplification procedures; ii) abundance of dengue vector during monsoon and dry seasons in the same households; iii) self-risk perception by the community members; and iv) human organizations responsible for interventions. Data included in the analysis are: a) two vector surveys [i.e., pupal surveys conducted in 847 households (monsoon season 2011) and 459 households (dry season 2012)]; b) two serosurveys [i.e., serosurveys conducted in 1128 households (pre monsoon season 2012) and 1130 households (630 paired sera and 500 replacement sera during post monsoon season 2012)]; c) socio-demographic survey of 300 households; and d) 12 focus group discussions and eight key informant interviews. Competent dengue vectors were detected in >40% and 12% of households during the monsoon and dry seasons respectively. The monsoon and dry seasonal pupal index were 0.40 and 0.33 respectively for the selected 12 wards. 80% IgG and 17% IgM were positive during pre monsoon serosurvey. Among the IgM positives, in-house PRNTs, using a serial dilution of sera mixed with a DENV serotype, are being carried out. There are significant variations in dengue risk perception between lower (low and medium) and higher socioeconomic groups. Also, experts ranked dengue risk at a much lower level than lay persons and experts emphasized the need for stronger institutional measures to control dengue outbreaks. In conclusion, the overall findings of the study will contribute to the advancement of DENV transmission knowledge, and will further the global knowledge of DENV epidemic potential.

107

HOSPITAL SURVEILLANCE FOR SYMPTOMATIC DENGUE INFECTION IN A REFERRAL HOSPITAL IN LIMA, PERU

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Dengue is a major public health problem in Peru and worldwide. Lima is considered an endemic area where autochthonous cases have been diagnosed every year. However many primary health practioners are not aware of this situation. We have reviewed dengue surveillance data to describe the clinical-epidemiological characteristics of suspected dengue cases that arrived to Cayetano Heredia Hospital from January 2011 to December 2012. Suspected dengue cases were defined as those coming from areas with known dengue transmission or infested with *Aedes aegypti* who had fever, and presented at least two or more of the following symptoms: headache, retro-orbital pain, myalgia, arthralgia and rash.Acute and convalescent blood samples were drawn and tested using a combination of serology (IgM and IgG) and molecular methods (PCR). 178 dengue suspect cases were reported during the study period, of these 53 (29.7%) had positive result for dengue infection. Dengue confirmed cases were predominantly males (58.5%), had a median age was 31 years [5-77]. 18.9% (n=13) patients didn't report traveling outside of Lima. Clinical information showed that 94.4% (n=50) reported headache, 88.7% (n=47) arthralgia, 83% (n=44) myalgia, 73.6% (n=39) lumbar pain, 71.7%(n=38) retro-ocular pain, and 47.7% (n=25) rash. Cases were more frequently diagnosed in the months of January (20.7%),February (15.1 %) and April (16.9%), corresponding to the summer time in Lima. This data confirms that dengue is currently circulating in Lima, both with imported and autochthonous cases occurring. The small number of cases detected probably corresponds to underreporting or because cases are not referred to a national hospital. The small proportion of dengue confirmed cases is probably related to the low specificity of the case definition. Clinical symptoms are similar to those reported before in the literature. We emphasize the importance of strengthening surveillance to better understand the epidemiology of autochthonous dengue transmission in Lima.

108

DENGUE IN PAN AMERICA 2001-2010: TRENDS IN IMPORTED CASES INTO THE UNITED STATES

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Dengue (DEN) fever is currently the most diagnosed traveler-related illness and there are 50-100 million global cases per year. An understanding of human travel patterns between DEN-endemic countries and the United States (U.S.) will improve risk assessments and identify potential routes of entry for DEN virus (DENV). As international travel increases, the geographic range of the four DENV serotypes may also increase. This intensifies the likelihood of multi-serotype epidemics that could impact public health. Travel statistics for 51 Pan American countries were analyzed from the Compendium of Tourism Statistics for 2001-2010. Countries were categorized by geographical region (i.e. North America, Central America, Andean, Southern Cone, Hispanic Caribbean, English, French and Dutch Caribbean). Most Pan American DEN cases occurred in Brazil (Southern Cone Region) in 2010 (> 1 million reported cases). In the U.S., the highest numbers of DEN infections were observed in travelers that visited the Dominican Republic (Hispanic Caribbean Region). Differences were observed in the annual numbers of DEN cases imported into the U.S. from different Pan American regions ($P < 0.05$). Variation in U.S. case reporting requirements between years were observed and this will be

discussed. Risk assessments showing the impact of travel on importation of DENV are essential to understand the role of human travel in pathogen spread.

109

SIGNIFICANT INCREASED NS1 ELISA SENSITIVITY DUE TO IMMUNE-COMPLEX DISSOCIATION IN DENGUE VIRUS TYPE 4 CASES IN BRAZIL

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In Brazil, dengue became a public health problem after the introduction of DENV-1 in 1986. In July of 2010, DENV-4 was isolated in Roraima. The detection of DENV NS1 as an alternative method useful for the early diagnosis of dengue has been shown. However, in secondary infections NS1 is less likely to be available for capture in an immunoassay due to immune-complex formation. We aimed to analyze the NS1 ELISA sensitivity for early diagnosis of DENV-4 cases recently reported and aiming to improve the sensitivity, results were compared to those by dissociating immune complexes from primary and secondary cases. DENV-4 sera (n=471) confirmed by virus isolation and/or were analyzed. The IgG_ELISA was performed immune response characterization. The Panbio dengue IgM Capture ELISA was used for the qualitative detection of anti-DENV IgM antibodies in serum for case confirmation The Platelia™ Dengue NS1 Ag-ELISA was used for NS1 capture. To improve the test sensitivity, two dissociation protocols were used: acid (AD) and heat-meadiated (HD). The overall NS1 antigen ELISA sensitivity in DENV-4 cases was 46.92% (221/471). Higher DENV-4 confirmation rates were observed when the NS1 assay was combined to MAC-ELISA (51.38%, p= 0,192), virus isolation (67.09%, p<0,05) or RT-PCR (99.57%, p<0,05).The 250 NS1 negative samples were submitted to the dissociation procedures and, 111/250 (44.4%) were positive after the AD procedure and 144/250 (57.6%) after the HD one. Positive NS1 was observed in 54.38% of primary and 39.07% of secondary cases. After the HD procedure, a significant NS1 sensitivity increase was observed in primary (82.01%) and secondary cases (73.10%, p=0,002).The NS1 assay results should be interpreted with caution when used alone due to the false negative results and the addition of a HD step prior to the assay to improve the sensitivity on endemic areas where secondary infections are more frequently reported is suggested.

110

DETECTION OF DENGUE VIRUSES IN *AEDES* MOSQUITOES FROM DIFFERENT LOCALITIES OF LAHORE, PAKISTAN

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Dengue viruses are transmitted through the bites of female *Aedes* mosquitoes to human mostly in urban areas of tropical/sub-tropical countries. Dengue epidemics are annually occurring in Pakistan since 2006. Recently in 2011 dengue became severe epidemic in province Punjab, where >15000 positive cases and >7300 deaths occurred, especially in the highly populated urban city of Lahore. With neither vaccine nor proper treatment for dengue, prevention of the disease depends upon the surveillance and early diagnosis/detection of dengue virus antigens from mosquito vectors which will serves as early warning system for forecasting impending outbreaks. In current study 28 entomological surveys were carried out in various localities of Lahore from March-September, 2011 for the collection of *Aedes* mosquitoes. Two species *Aedes aegypti* and *Ae. albopictus* were found commonly during this period. However, *Ae. aegypti* were present throughout these months while *Ae. albopictus* appeared in the months of July-August, 2011. In addition various types of natural and artificial breeding containers were also observed for immature stages of *Aedes* mosquitoes in all localities visited during above mentioned period. The most productive containers were automobiles used tyres for larval production with 94% positivity. Collected mosquitoes were screened for dengue viruses using dengue specific monoclonal antibodies (MAB) as antigen capture Enzyme Linked Immunosorbant Assays (ELISAs). Of the 114 pools of *Ae. aegypti* females (n=570) screened, 31 pools were found positive for dengue viruses indicating 27.19% infection rate (MIR). However, of the 04 pools of *Ae. albopictus* females (n=20) screened; only 1 pool was found positive with 25% infection rate (MIR). This is the first report of DENV detection from adult females of *Ae. aegypti* and *Ae. albopictus* collected from different localities of Lahore, Pakistan.

111

DENGUE SEROTYPE-SPECIFIC DIFFERENCES IN CLINICAL MANIFESTATION, HEMATOLOGICAL PARAMETERS, PLASMA VIRAL CONCENTRATION AND RISK OF SEVERE DISEASE IN ADULTS

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Studies on serotype specific clinical manifestations of dengue and disease severity on adults are limited. Furthermore, the DENV-2 genotype in previous studies is the reportedly more severe Asian genotype rather than the cosmopolitan genotype circulating in Singapore and neighbouring countries whose clinical manifestations and virulence is still undocumented. We prospectively recruited adult febrile patients without alternate diagnosis to dengue from April 2005 to December 2011 from primary care and a tertiary hospital in Singapore. These cases were followed up with detailed clinical and laboratory data. Outcomes were defined using both the World Health Organization 1997 and 2009 criteria; dengue hemorrhagic fever (DHF) and severe dengue (SD). Infecting serotype was identified in 469 dengue confirmed patients comprising 22.0% DENV-1, 57.1% DENV-2, 17.1% DENV-3 and 3.8% DENV-4. After adjusting for potential confounders, cases infected with DENV-1 were more likely to present with red eyes (adjusted relative risk [aRR] 1.61, 95%CI 1.13 to 2.29) while absence of red eyes (aRR 0.74, 95% confidence interval [CI] 0.60 to 0.92) but presence of joint pain (aRR 1.19, 95%CI 1.04 to 1.35) and lower median platelet count was associated with DENV-2 cases. DENV-1 was associated with both DHF (aRR 1.74, 95%CI 1.1 to 2.7) and SD (aRR 2.1, 95%CI 1. 1 to 4) while DENV-2 had a lower risk of DHF (aRR 0.5 95%CI 0.35 to 0.75). We also found DENV-1 cases to have a higher plasma viral RNA concentration compared to DENV-2 and DENV-3 (aRR: 1.7, 95%CI 1.29-2.15). Available genotype data show that 95% of DENV-1 cases are genotype 1 while 100% of DENV-2 cases are cosmopolitan. DENV-1 infections were more likely to develop severe disease compared to DENV-2 infections amongst adults in Singapore. Differences in dengue serotype outcome may be associated with viral load. Our results suggest that genotype differences within serotypes may alter virulence highlighting the importance of molecular surveillance.

112

DENGUE VIRUS INFECTION AMONG HAITIAN AND EXPATIRATE NON-GOVERNMENTAL ORGANIZATION WORKERS - HAITI, 2012

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Dengue is a mosquito-borne infectious disease endemic throughout the tropics and subtropics; however, little is known about dengue in Haiti. In October 2012, the Haitian Ministry of Public Health and Population and CDC-Haiti was notified of 25 non-governmental organization (NGO) workers near Léogâne with dengue, including six who required medical evacuation. To estimate the incidence of recent dengue virus (DENV) infection and identify risk factors for infection among Haitian and expatriate NGO workers in Léogâne and Port-au-Prince, we conducted a seroincidence study wherein we administered questionnaires and collected blood samples; conducted an entomological investigation in 100 premises around work sites and residences of participating NGO workers; and distributed educational material. Sera were tested at CDC Dengue Branch by ELISA to detect anti-DENV IgM antibody and by RT-PCR to detect DENV nucleic acid. Of 181 NGO workers surveyed, 76% were male and 71% were Haitian; median age was 40 years (range: 19-66). Most (93%) used mosquito-bite avoidance practices. Of 173 (96%) participants who provided a blood sample, none had DENV detected by RT-PCR. Seventeen (10%; 8 expatriates and 9 Haitians) had detectable anti-DENV IgM, indicating recent DENV infection; of these, 6 (35%) reported a febrile illness during that time period. Participants reporting a history of asthma (Odds ratio [OR]=9.3, 95% confidence interval [CI] =2.2-38.9) or working near open water sources (OR=3.6, 95% CI=1.3-10.1) were more likely to be IgM anti-DENV positive. Of 254 mosquito pupae that were collected and identified, the most abundant mosquito species was *Aedes aegypti* (65%), followed by *Aedes albopictus* (27%). Sixty-one percentage of home sites had at least one container with mosquito larvae. This investigation revealed high rates of DENV infection and a high density of *Aedes* container mosquitoes in the Léogâne area. Both Haitians and expatriates working in Haiti should be made aware of dengue and encouraged to take appropriate personal protective measures to avoid mosquito bites.

113

IMPROVING THE SPECIFICITY OF A COMMERCIAL ANTI-DENGUE IGG IMMUNOASSAY BY CUTOFF VALUE OPTIMIZATION

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Serosurveys to estimate the exposure of a population to various pathogens often use IgG as a marker for prior exposure due to its continued persistence years after exposure. However, enzyme-linked immunosorbent assays (ELISAs) employed for the detection of anti-dengue IgG are notoriously cross-reactive with other flaviviruses, often resulting in poor assay specificity and consequent limitations in the context of seroepidemiological studies. In this study, we compared the specificity of a commercially available anti-dengue IgG ELISA (Focus Diagnostics) with the most specific serological standard: the Plaque Reduction Neutralization Test (PRNT). Using sera from traveling military members and their dependents, 17 of 36 IgG ELISA positive specimens (classified using the manufacturer's recommended cut-off of Index Value = 1) did not have measurable neutralizing antibody titers, resulting in a specificity of only 52%. By increasing the cut-off to an Index Value of at least 2.25, the apparent specificity with respect to PRNT can be increased to 76.5% while maintaining a sensitivity of 78.9%. We also found that specimens with a monotypic neutralizing response via PRNT (consistent with primary infection) had significantly lower average Index Values (t-test, $p<0.02$) than those demonstrating a polytypic response (range 1.60-2.32 versus 1.94-5.16, respectively). This suggests that increasing the threshold to positivity in an effort to reduce false positive results might inadvertently reduce the sensitivity of the assay for primary infections. Overall, our data indicate that dengue seropositivity may be overestimated when using the ELISA alone according to the manufacturer's protocol. We conclude that the Index Value of the commercially available IgG ELISA should be optimized according to the endemicity of the target population prior to initiating a study, in order to achieve the desired balance between specificity and sensitivity.

114

EVALUATION OF A DENGUE RAPID DIAGNOSTIC TEST USED DURING A DENGUE OUTBREAK - REPUBLIC OF THE MARSHALL ISLANDS, 2011-2012

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Dengue is a mosquito-transmitted infectious disease endemic throughout the tropics. The Dengue Duo rapid diagnostic test (RDT), which detects dengue virus (DENV) nonstructural protein 1 (NS1) and anti-DENV IgM (IgM), has a reported sensitivity and specificity ≥85%. In October 2011 a dengue outbreak was identified in the Republic of the Marshall Islands, a small Pacific island country with a population of 53,158. The RDT was used to identify cases and describe the epidemiology of dengue in RMI. Most specimen tested with the RDT were sent to Centers for Disease Control and Prevention Dengue Branch (CDC) for confirmatory testing and to evaluate the in-field performance of the RDT. Lab-positive cases had NS1 or IgM detected with the RDT, or DENV nucleic acid or IgM detected at CDC by RT-PCR or IgM ELISA, respectively. 1,603 suspected dengue cases (3% of RMI residents) were identified during the outbreak, of which 867 (51.1%) were lab-positive. Only DENV-4 was detected during the outbreak, and phylogenetic analysis of the envelope gene showed that it belonged to Clade II of the Indonesian genogroup. Individuals 15-29 years of age were most affected and tested lab-positive at a rate of 32.5 per 1,000 residents. Other clinical presentations included vertical DENV transmission (n=2; 1% of all mothers that delivered during the outbreak), DENV/*Salmonella* Typhi co-infection (n=2), DENV/*Mycobacterium leprae* co-infection (n=1), and dengue encephalitis (n=1). There were no dengue-related deaths. Confirmatory testing of 705 individuals tested with the RDT showed a sensitivity of 66.1% and specificity of 83.3%. The lower-than-reported performance of the RDT could be due to prospective use in the field, and/or the cause of the outbreak being DENV-4, which has not been consistently included in prior RDT evaluations. Although the RDT had less than ideal sensitivity, it provided the means to detect the outbreak, follow

its progression, and determine some of its epidemiologic characteristics. Further field studies are needed to determine the utility of RDTs in resource poor, dengue endemic areas.

115

CATCHING PROTEUS: THE DEVELOPMENT AND CLINICAL EVALUATION OF HIGHLY SENSITIVE DENGUE DIAGNOSTICS

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Dengue virus (DENV) is the most common vector-borne pathogen worldwide; infection with one of four related serotypes results in a range of clinical manifestations spanning minimally symptomatic infection, dengue fever, and severe dengue. Given such protean clinical manifestations, diagnosis relies on the use of accurate laboratory tests. A number of nucleic acid amplification tests have been developed for this purpose, though direct method comparisons are rare. Furthermore, patients with severe dengue may present later in the disease course, but such patients are often excluded from assay validation studies. We report the design and evaluation of two real-time RT-PCR (rRT-PCR) assays for the detection of DENV, including a single-reaction, serotype-specific rRT-PCR (the multiplex assay), and an internally-controlled rRT-PCR for pan-DENV detection (the pan-DENV assay). The linear range for both assays, established with quantified plasmid DNA and genomic RNA from control strains of all four serotypes, extends from 1.0 to 7.0 log10 complementary DNA (cDNA) equivalents/µL. The lower limits of 95% detection are between 0.51 and 7.75 cDNA equivalents/ µL, depending on the serotype. A clinical evaluation was then performed using 199 plasma samples from suspected dengue cases in Nicaragua (n=160) and Sri Lanka (n=39) who presented between two and nine days after illness onset. All samples were tested using the multiplex and pan-DENV assays as well as a widely-used, hemi-nested RT-PCR and the FDA-approved CDC DENV-1-4 rRT-PCR. Both the multiplex and pan-DENV assays proved significantly more sensitive than either comparator ($p \leq 0.01$ for all comparisons), with equivalent specificity. The improved clinical performance of these assays resulted from the maintenance of sensitivity in samples collected on or after day-of-illness five and from patients with detectable anti-DENV IgM at presentation. The ability to confirm the diagnosis of dengue later in the disease course has the potential to improve dengue management, patient outcomes, and epidemiologic surveillance.

116

SEQUENTIAL INFECTION WITH DENGUE AND INFLUENZA VIRUSES AFFECTS DISEASE SEVERITY IN MICE

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Both influenza and dengue are major public health problems worldwide. In 2009, Nicaragua experienced a severe dengue epidemic, marked by atypical clinical presentation with early onset of compensated shock and poor peripheral perfusion, as we observed in two studies of dengue in Managua. Multivariate analysis revealed only the year 2009 as a significant risk factor, and neither the dominant dengue virus (DENV) serotype nor virus clade changed from 2008 to 2011. Our parallel influenza cohort study and national surveillance data showed that in 2009 the influenza A-H1N1 (H1N1) pandemic and dengue epidemic overlapped for 8-10 weeks. We hypothesized that sequential or co-infection of H1N1 and DENV modulates host responses and leads to more severe disease, and we found increased risk of dengue shock among Nicaraguan children with anti-H1N1 antibodies in 2009. Guided by these observations, we established a mouse model of sequential infection to explore the mechanism of interaction between the two viruses. We used the pandemic H1N1 isolate A/NI/5227/2009 from Nicaragua (intranasal), expanded in embryonated chicken eggs, and the virulent DENV2 strain D220 (intravenous), which was obtained by serial passaging of a clinical DENV2 isolate between the serum of immunodeficient mice and mosquito cells and contains several defined mutations that enable the virus to persist longer peripherally before clearance. It is known that DENV blocks IFN-α/β signaling pathways in DENV-infected human cells, but not in mice. As a result, DENV does not efficiently replicate or cause disease in C57BL/6 wild-type (WT) mice. However, DENV-infected C57BL/6 mice deficient in the IFN-α/β receptor (*Ifnar*$^{-/-}$) develop a lethal vascular leak syndrome with features of severe dengue disease in humans. We show here that sequential inoculation of *Ifnar*$^{-/-}$ mice with both viruses within 2 days of each other causes lethal disease at doses that lead to sublethal disease after infection with only one virus. We are currently investigating sequential inoculation of WT mice, as well as immunological pathways involved in the interaction between the two viruses by studying tissue viral load and gene expression profiles in both *Ifnar*$^{-/-}$ and WT mice. This study may reveal new mechanisms of immune regulation and may inform future vaccine strategies in endemic areas where both viruses co-circulate.

117

EARLY DETECTION OF PRE-SYMPTOMATIC DENGUE VIRUS INFECTION FROM GEOGRAPHIC CLUSTER INVESTIGATIONS IN KAMPHAENG PHET, THAILAND

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A geographic cluster study using hospitalized dengue index cases was conducted in Kamphaeng Phet province, Thailand from November 2009 to December 2011. We aimed to demonstrate the early detection of pre-symptomatic dengue virus (DENV) infections. Contact subjects were enrolled from the same house as the index case and from houses within 200 meters that had one or more occupants with a history of fever during the preceding 7 days. From 203 index cases, 889 contacts were enrolled of which 365 reported symptoms and 524 reported no symptoms at enrollment (day 0). Of the 365 symptomatic contacts on day 0, 136 (37.3%) had positive IgM/IgG ELISA on day 0 and/or day 15. Of the 524 contacts without symptoms on day 0, 67 (12.8%) had positive IgM/IgG ELISA. Characteristics of these 67 cases included female: male ratio of 1.6:1 and average age of 32.2 years (range 3.5-82). Twelve (17.9%) were PCR-positive on day 0 including 1 DENV-1, 5 DENV-2 and 6 DENV-3, while 55 were PCR-negative. There were 12 acute primary infections, 49 acute secondary infections and 6 recent secondary infections. Forty-five (67.2%) of these 67 were from index houses, 8 (11.9%) from houses within 50 meters of the index house, 10 (14.9%) from houses between

50 and 100 meters, and 4 (6.0%) between 100 and 150 meters. Fifteen cases that were initially without symptoms developed fever during the 15 (+/- 5) day follow up period (range 1-19 days). Seven of these 15 "pre-symptomatic" cases were hospitalized including 3 with DF and 4 with DHF grade II. PCR testing of blood samples collected on the day of fever onset showed 3 DENV-1, 8 DENV-2, 1 DENV-3 and 3 negative. Fourteen of 15 had acute secondary infection. Our study demonstrated an overall DENV infection rate of 22.8% around hospitalized DENV-infected index cases. Of the DENV-infected contacts, 7.4% were pre-symptomatic with almost half requiring subsequent hospitalization. Our study demonstrates that close monitoring near hospitalized dengue cases allow for pre-illness detection of DENV infections for early immunological studies and/or clinical intervention.

118

MOLECULAR EPIDEMIOLOGY OF DENGUE VIRUS CIRCULATING AMONG BLOOD DONORS IN PUERTO RICO, 2012-2013

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Dengue viruses (DENV-1 to -4) are flaviviruses primarily transmitted by mosquitoes; however, these viruses have been shown to be transmissible by blood transfusion. DENV is endemic in Puerto Rico (PR), where all four types have circulated causing periodic epidemics, with predominance of DENV types varying from epidemic to epidemic. In 2010, PR underwent the largest dengue epidemic in history, with predominant circulation of DENV-1 and DENV-4. Phylogenetic studies conducted using the envelope protein gene (E) from PR isolates during 2010, showed that the DENV-1 strains circulating that year differed from strains that had previously circulated in the island, suggesting that *in situ* evolution had occurred for DENV-1. The studied DENV-4 strains from 2010 were found to be closely related to those reported circulating, for at least the last decade in the island. The aim of this study was to analyze the genetic makeup of DENV circulating in PR in 2012-2013. The study included surplus blood specimens from 36 blood donors that tested reactive for DENV RNA by an investigational TMA assay used by the American Red Cross, from August 2012 to April 2013. All 36 specimens were tested at the FDA for DENV RNA by an in-house TaqMan RT-PCR assay. Sixteen of the 36 specimens yielded high viral RNA titers as detected by TaqMan in the plasma or whole blood samples, 14 of which were identified as DENV-1, while 2 were identified as DENV-4. Those 16 specimens were subjected to amplification of the complete E gene by RT-PCR directly from the plasma or whole blood samples or from the supernatants obtained from the first passage in mosquito C6/36 cells, and PCR products were subjected to bidirectional Sanger sequencing. Sequence data was subjected to phylogenetic analysis by maximum-likelihood and Bayesian methods. The results of the analysis revealed that the newest DENV-1 clustered within clade "c" of genotype V and associated with those previously identified circulating in 2010. The 2012 DENV-4 strains (genotype II) were found to be closely associated to strains ARC-19-10 and ARC-42-10 that were previously reported in studies from the 2010 dengue epidemic in PR. These results revealed that the genetic makeup of DENV-1 and DENV-4 in PR during 2012-2013 remained unchanged from that found in viruses circulating during the 2010 epidemic.

119

GENETIC RELATEDNESS OF TRAVELER-ASSOCIATED DENGUE VIRUS AND DIVERGENCE OF NEW LINEAGES IN THE AMERICAS

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The increase in travel and trade in the past 30 years has contributed to the global transmission of dengue viruses (DENV) and increased risk of virus importation and infection of susceptible populations. Dengue is a leading cause of febrile illness in travelers returning from the tropics and sub-tropics. In the United States more than 15,000 travelers require dengue diagnostic testing every year. Infected travelers have the potential to initiate endemic DENV transmission in regions where the *Aedes* mosquito vector is present. Several dengue outbreaks have been reported in the Americas during 2009-2012 including clusters associated with travel from endemic countries to areas of the US where dengue has not been previously transmitted. Phylogenetic and relatedness analyses using maximum likelihood and Bayesian probability performed on cases reported from Nebraska and Georgia indicated importation from a recent DENV-1 epidemic in Haiti. A similar analysis of cases from Key West, Florida indicated an importation of DENV-1 led to emergence of a distinct monophyletic sublineage not previously detected in the US. A further large-scale phylogenetic analysis of the DENV-1 American-Asian genotype transmitted in the Americas revealed the occurrence of a significant lineage turnover estimated to have occurred in 1998 and the emergence of two significantly distinct lineages. One lineage is associated with strains predominantly transmitted in South America and the Caribbean basin; associated with the Nebraska and Georgia cases. The second lineage is associated with strains transmitted in Central America and has recently diverged into sublineages affecting non-endemic areas like Key West and regions of northern Mexico. Fluctuations in the relative genetic diversity of the American DENV-1 were detected using Bayesian MCMC and seem to correlate with epidemiological observations. This study reveals the divergence of the DENV-1 American-African genotype into two major lineages with evolutionary dynamics associated with distinct geographical regions. Widespread DENV transmission facilitated by frequent travel presents a potential driver of evolution exposing the virus to new environmental selection pressures and host populations.

120

COMPARISON OF ANTI DENV/JEV IGG-MONOCLONAL ANTIBODY ENZYME-LINKED IMMUNOSORBENT ASSAY (IGG-MAB ELISA) AND HEMAGGLUTINATION INHIBITION ASSAY (HAI)

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Dengue virus (DENV) infection can manifest as clinically inapparent, undifferentiated febrile illness, classical dengue fever, or dengue hemorrhagic fever. The majority of DENV infections in children are thought to be subclinical. In our prior prospective cohort studies on the epidemiology of inapparent and symptomatic acute DENV infections in children in Kamphangphet, Thailand, we tested pre- and post-surveillance period serum samples using dengue hemagglutination inhibition assay (HAI) for screening and plaque reduction neutralization test (PRNT) for confirmation. These two methods have been the most commonly used in serological diagnosis, but they can be time and resource intensive. Here, we seek a more rapid and practical assay that may be used to replace HAI for screening. Pre- and post-surveillance blood samples collected from prior child cohort studies in Kamphaeng Phet, Thailand underwent testing in order to compare an anti-DENV/JEV IgG-MAb ELISA with dengue HAI. Tested sera consisted of 91 serum pairs with DENV or JEV infection, and 91 serum pairs with no flavivirus infection based on HAI assay. Monoclonal antibodies used in the anti-DENV/JEV IgG-MAb ELISA were 2H2 and J93

MAbs, which were specifically captured to inactivated DENV and JEV antigen, respectively. Using two serum dilutions (1:400 and 1:1600), a difference in OD of ≥0.200 between sera pairs was used to define seroconversion in the ELISA. When compared with HAI, the anti DENV/JEV IgG-MAb ELISA showed 90% sensitivity (82/91 HAI-positive pairs) and 100% specificity (91/91 HAI-negative pairs). Our results support the potential use of anti-DENV/JEV IgG-MAb ELISA for serological screening for DENV infection.

121

LOW SERUM CHOLESTEROL AND GALLBLADDER WALL THICKENING AS PREDICTIVE MARKERS FOR SHOCK IN DENGUE PATIENTS

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Dengue is the most prevalent mosquito-borne viral disease of humans worldwide. Dengue virus (DENV) infection leads to a range of outcomes, including asymptomatic infections, undifferentiated febrile illness, classic dengue fever, and severe, life-threatening syndromes. Shock represents the most common severe manifestation in dengue patients, especially children, and predicting its occurrence is a challenge for physicians. Previous studies have described that low serum cholesterol and gallbladder wall thickening are associated with severe dengue. Here, we analyzed cholesterol levels and gallbladder wall thickening as predictive factors for shock in pediatric dengue patients using data from seven years (2005-2012) of a hospital-based study in Managua, Nicaragua. A total of 506 laboratory-confirmed dengue cases were included. Before the onset of shock, cholesterol levels were measured in serum, and gallbladder wall thickness was assessed by ultrasonography. The frequency of shock was significantly higher in patients who initially exhibited low cholesterol levels (<100mg/dl) compared to patients with normal cholesterol levels (10.4% versus 4.6%, p=0.020). In children with gallbladder wall thickening (>3mm), subsequent occurrence of shock was also higher compared to children with a normal gallbladder wall thickness (14.0% versus 5.6%, p=0.006). When adjusted for day of measurement, the relative risk (RR) for shock was 2.58 (95% confidence interval [95%CI] 1.18-5.61) in children with low cholesterol levels. In children with gallbladder wall thickening >3mm, the RR was 2.94 (95%CI 1.60-5.41). A predictive model was constructed using cholesterol level, gallbladder wall thickening and day of measurement. Sex, age, obesity, the presence of a chronic disease, primary/secondary DENV infection and DENV serotype were not associated with shock in this model. The area under the curve of the receiver operating characteristic (ROC) analysis of the model was 0.75 (95%CI 0.66-0.83) and the model Brier score was 0.068, indicating that the model is useful and provides accurate probabilistic predictions, respectively. Our results suggest that cholesterol levels and gallbladder wall thickening can be used as predictive factors for shock in dengue patients. Validation of this model using prospectively collected data is currently underway.

122

DENGUE VIRUS NONSTRUCTURAL PROTEIN 1 VACCINE PROTECTS AGAINST LETHAL CHALLENGE IN A DENGUE MOUSE MODEL

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Dengue virus (DENV) is a mosquito-borne flavivirus that causes an estimated 100 million cases of dengue and approximately ~500,000 hospitalizations annually. A subset of dengue cases progress to a vascular leak syndrome that can have high case fatality rates in the absence of appropriate and timely treatment. DENV nonstructural protein 1 (NS1) is secreted by infected cells and is found at high levels in patient serum during acute illness. To investigate the potential of NS1 as a vaccine candidate, we examined the protective efficacy of immunization with recombinant NS1 protein against lethal DENV infection in a mouse model of a vascular leak syndrome with features of severe dengue disease in humans. Interferon alpha/beta receptor-deficient C57BL/6 mice were injected intraperitoneally 3 times over an 8-week period with 20 ug of recombinant NS1 combined with different adjuvants, including alum, Sigma adjuvant system (SAS), CpG DNA, MF59 and/or monophosphoryl lipid A (MPLA). Two weeks after the third immunization, mice vaccinated with NS1 combined with either SAS and CpG or MPLA and MF59 were protected against a lethal peripheral challenge with DENV2. Vaccination with NS1 combined with alum and/or CpG DNA alone provided no protection against DENV2 challenge. All vaccinated groups demonstrated comparable levels of total anti-NS1 IgG; however, mice protected against lethal challenge had higher levels of IgG2b antibodies specific for NS1. We are currently investigating the possible mechanisms of antibody-mediated protection, including antibody-dependent cytotoxicity and inhibition of vascular leak. We are also exploring the potential role of $CD4^+$ T helper cells and $CD8^+$ cytotoxic T cells against NS1 in protection. Thus, immune responses to a DENV nonstructural protein can provide protection against severe disease and recombinant NS1 may provide an alternative vaccine against dengue.

123

THE GREEN WAY (CAMINO VERDE) TO DENGUE PREVENTION THROUGH EVIDENCE-BASED COMMUNITY MOBILIZATION: A CLUSTER-RANDOMIZED CONTROLLED TRIAL IN NICARAGUA AND MEXICO

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Dengue, a mosquito-borne viral disease, is a significant health, economic, and social burden worldwide. As no vaccine or therapeutic exists, prevention relies on government-led control of the mosquito vector, based mainly on larvicide and insecticides. To demonstrate that community mobilization driven by feedback of evidence is as least as effective as chemical approaches to reduce *Aedes aegypti* breeding sites and entomological indices, we conducted parallel cluster-randomized controlled trials in Managua, Nicaragua, and Guerrero, México, from June 2010 to February 2013. After random selection of eligible clusters (~140

households each), we conducted a two-phased baseline measurement (pre- and post-dengue season) using questionnaires, entomological inspections, and saliva samples (for dengue serology). A further randomization into intervention vs. control clusters was also stratified by key parameters. In Managua, clusters in 30 neighborhoods received the intervention with an equal number as controls. In Guerrero, 90 clusters were randomized to 15 intervention and 15 control clusters in each of 3 regions: mostly-urban Acapulco and mostly-rural Costa Grande and Costa Chica. We used entomological and serological indices to measure primary outcomes, while secondary outcomes included social capital. The intervention, called SEPA (Socialization of Evidence for Participatory Action), occurred at 3 levels: household, neighborhood and inter-neighborhood. SEPA emphasizes informed dialogue and socialization of evidence to develop locally relevant and autonomous interventions based on common processes. We conducted impact measurement of *Aedes* indices, dengue infection and secondary outcomes in Aug 2012-Feb 2013, with >20,000 household visits, ~10,500 paired saliva samples and ~75,000 container inspections among all sites. The results show a significant effect of SEPA in entomological terms as seen in ~40% reduction of *Aedes* pupal indices in intervention sites in both countries. In contrast, the larvicide program had no effect in reducing *Aedes* house indices, and in water storage barrels was less effective than scrubbing or using lids. A significant reduction in indices of anti-dengue antibodies in children's saliva was observed as well. These results show that community-led interventions to reduce dengue risk are workable and offer governments further sustainable options in their efforts to control dengue.

124

DENGUE IN THE ELDERLY IN SINGAPORE

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Increasingly dengue occurs in older children and young adults. In Singapore, dengue in the elderly accounted disproportionately for a majority of dengue death. We aim to compare clinical features and World Health Organization (WHO) 1997 and 2009 categories, and outcomes between adult dengue patients < and ≥60 years old, and explore the impact of co-morbidity and nosocomial infections on clinical outcomes in the elderly. Patients with positive dengue polymerase chain reaction (PCR) or who fulfilled WHO 1997/2009 probable dengue criteria with positive dengue serology at Communicable Disease Centre, Singapore, from 2005 to 2008 were studied. Of 7735 cases, 349 were ≥60 years, and PCR was positive in 26%. The elderly suffered more dengue hemorrhagic fever (DHF) (30% vs. 22%) and severe dengue (SD) (21% vs. 15%) ($p<0.05$). The elderly were less likely to fulfill WHO 1997 (78% vs. 89%) ($p<0.05$), but not WHO 2009 probable dengue (74% vs. 71%). Time to dengue diagnosis was similar. The elderly had more malaise (35% vs. 28%) and hepatomegaly (3% vs. 1%), and less mucosal bleeding (12% vs. 24%) ($p<0.05$), but were similar in other warning signs. Intensive care admission occurred in 21 and death in 6, with no age difference. Notably, the elderly stayed in hospital longer (median 5 vs. 4 days), and suffered more pneumonia (4.2% vs. 0.6%) and urinary infection (1.9% vs. 0.3%) ($p<0.05$). Independent predictors of excess hospital stay were age (adjusted odds ratio [aOR] 2.0, 95% confidence interval [CI] 1.4-2.8), critical illness (aOR 5.0, 95%CI 2.6-9.6), nosocomial infection (aOR 11.0, 95%CI 7.0-18.0), Charlson's score (aOR 5.0,95%CI 2.2-17.0) and DHF/SD (aOR 2.2, 95%CI 1.8-2.7). Older dengue patients presented more atypically, and suffered more DHF and SD, and nosocomial infections. Aside from dengue severity, age, co-morbidity and nosocomial infection were independently associated with longer hospital stay.

125

DIFFERENTIAL PATTERNS OF INTRAHOST DENGUE VIRUS DIVERSITY IN PRIMARY AND SECONDARY HUMAN DENGUE VIRUS INFECTIONS

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Dengue virus (DENV) is a mosquito-borne *Flavivirus* with a positive-strand, nonsegmented RNA genome that is responsible for up to 400 million infections and 100 million cases of dengue worldwide every year. Genetic variation associated with intrahost DENV populations has been postulated to influence viral fitness and disease pathogenesis. We previously reported on the use of a whole-genome amplification approach coupled with deep sequencing to capture intrahost diversity across the entire coding region of DENV-2 (Parameswaran P *et al.*, *J Virol*, 2012). Using a similar approach, we have now sequenced DENV-3 genomes from the PBMCs and plasma of 67 Nicaraguan individuals enrolled in a hospital-based study in Managua with primary or secondary DENV infections and captured on average 92% of the DENV coding region in each sample (range 31.9-99.9%). We observed significant differences in intrahost diversity between genes, with the Envelope (E) gene exhibiting the highest incidence and abundance of intrahost variants. Differences were also discerned between the three antigenically distinct domains of E, with variants predominantly localized to E domain III (EDIII). Interestingly, overall incidence and abundance of intrahost variants in EDIII (but not other genes or E domains) was substantially higher in secondary as compared to primary DENV infections. We also identified a hotspot for diversity in the AB loop region in EDIII at residue 315, which is conserved across all flaviviruses and is presumed to play a role in viral-host membrane fusion in the endosome during infection. Variants at His-315 were observed in >80% of all dengue samples, with one viral variant (H315L) significantly more abundant in secondary dengue compared to primary dengue cases. We are currently assessing binding profiles and neutralizing/enhancing capabilities of pre-existing antibodies in individuals with primary and secondary dengue in order to understand the origin and phenotype of the AB loop variants. Our data thus illustrate that high-resolution mapping of viral intrahost diversity can be used to identify viral genomic hotspots for intrahost diversity in acute human dengue and presumably other short-lived viral infections. Further investigations into the nature of these hotspots will contribute to our understanding of selection pressures exerted by various immunological components of protection and disease in human dengue.

126

LABORATORY ANALYSIS OF ARBOVIRUS SURVEILLANCE IN SOUTHERN THAILAND

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Epidemiological information from SouthernThailand has considerable gaps. This region is of significant interest due to its location, and the proximity to and cross border traffic with Malaysia. Most of Southern Thailand is rural with many of the inhabitants living in rural and suburban areas, often working in the agricultural field and continuously exposed to mosquito vectors. Systemic arbovirus infection disease surveillance in the area has increased its effort after a big outbreak of chikungunya virus that infected 49,069 people, as reported in 2008-2009. The Armed Forces Research Institute of Medical Sciences (AFRIMS), in collaboration with Prince of Songkla University (PSU), conducted the collection and testing

of approximately 300 patients in Southern Thailand from July 2012 to Jun 2013 in an effort to identify and characterize chikungunya and other arboviruses currently circulating. Patients presenting dengue-like illness, with and without affecting joint pain, or symptoms consistent with viral encephalitis, or with undifferentiated febrile illnesses were enrolled and asked questions pertaining to their illness and environmental, living and working conditions. Surveillance activities included collection of close to 300 acute specimens during the time of illness and a second convalescent specimen10-21 days post. We present data using sensitive hemi-nested PCR conducted on each sample to identify dengue,Japanese encephalitis and chikungunya viruses circulating in the acute serum. Our data includes levels of acute and convalescent IgM and IgG antibody responses against the same pathogens and their neutralization levels using hemagluttinin inhibitory assay (HAI) against dengue, Japanese encephalitis and chikungunya viruses. The viral genome will be partially sequenced either directly from human sera or after amplification in culture.

127

EPIDEMIOLOGY AND PHYLOGENY OF DENGUE TYPE 2 VIRUSES RESPONSIBLE FOR THE 2011 OUTBREAK IN PANAMA

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After 50 years of absence, Dengue virus (DENV) reemerged in Panama in 1993. Since then, three epidemics have occurred in the country: 1993, 2005 and 2011. The first and the last ones were associated with the introduction of DENV-2 virus. The 2011 outbreak resulted in the highest mortality; 55% of all dengue-related deaths recorded in Panama since 1993, occurred in 2011. The 2011 outbreak was focused in the metropolitan area of Panama city and the San Miguelito district. A phylogenetic analysis was done of the coding of the E (envelope) protein of representative DENV-2 strains that circulated in Panama from 1993 to 2011. We found that all DENV-2 strains belonged to the Southeast Asian/American genotype and could be divided in four clades; each one was related to clades from the Caribbean (Panamanian clades from 1993-94), from South America (clades from 1999-2004) or from Central America (clades from 2011). Interestingly this last clade, responsible of the 2011 outbreak in Panama, has also been associated with more complicated cases of DENV-2 in Central America (Nicaragua and Guatemala) reported recently. This is the first time that DENV strains from Panama have been sequenced and analyzed phylogenetically, allowing us to characterize the genotypes of DENV-2 circulating in the country and their relation with other DENV-2 strains described in the region. Future studies are planned to sequence the complete genome of DENV-2 strains from each one of the clades to further characterize them and try to relate specific mutations with pathogenesis.

128

CHARACTERIZATION OF DENGUE FEVER IN ADULTS IN MEDELLIN, COLOMBIA

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In Colombia, all four dengue virus (DENV) serotypes circulate in many parts of the country. During the last 10 years, there has been a significant increase in the number of cases of dengue fever (DF) with almost 50,000 cases reported in 2012. Medellín, the second largest city in Colombia, is located in the foothills of the Aburra Valley, a region endemic for DENV with periodic DF outbreaks. In 2010, more than 25,000 cases (454 cases per 100,000 habitants) of DF and 300 cases (4.94 cases per 100,000 habitants) of dengue hemorrhagic fever were detected in the Aburra Valley. In the city of Medellín, there were over 17,000 cases of DF with an incidence of 745.4 per 100,000 habitants. In Colombia, epidemiological data indicate that adults are frequently infected with DENV and can play an important role in virus transmission and epidemiology. To study the epidemiology and immunologic factors impacting dengue disease in an adult population, we established a cohort of 2,026 adults for dengue surveillance in Medellín, Colombia. Upon the identification of a febrile illness in a cohort member, clinical information and acute and convalescent blood samples are collected and dengue diagnostic tests are performed. Cohort volunteers also participate in routine blood collections every six months that include processing for peripheral blood mononuclear cells. Complete results from the first year of surveillance will be presented.

129

DENGUE EPITOPE MAPPING AND VACCINE DISCOVERY USING A BACTERIPHAGE VIRUS-LIKE PARTICLE PLATFORM

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There are 4 recognized serotypes of Dengue virus (DENV1-4). Primary infection with DENV induces lasting immunity only to the infecting serotype, but secondary infection with a different serotype results in protection against all 4 serotypes. We hypothesized that broadly neutralizing convalescent serum from DENV secondary infection could be used to identify DENV envelope protein (E) linear epitopes that could be targeted with a pan-DENV vaccine. Our lab recently developed a novel affinity selection platform technology based on MS2 bacteriophage VLPs (MS2-VLPs). This platform combines the affinity selection capability of phage display with the high immunogenicity of VLPs, thus integrating the epitope identification and immunization functions into a single particle. We constructed an antigen fragment library in which overlapping 10 amino acid peptides from DENV-3 were displayed on the surface of MS2-VLPs. We carried out 2 rounds of affinity selection using broadly neutralizing IgG isolated from a Panamanian patient with secondary DENV infection. The resultant selectants were then sequenced using Ion Torrent deep sequencing in order to provide a comprehensive view of the antibody response to DENV linear epitopes. The most abundant linear E epitope mapped to the stem region of the protein (aa 393-403), which we have named stem-associated peptide (SAP). Comparative sequence analysis of E for all 4 serotypes of DENV show SAP to be highly conserved, and mapping to the 3D structures of E indicate SAP is likely exposed during viral fusion. In order to test the ability of MS2-VLPs displaying SAP (MS2-VLP-SAP) to induce a broadly neutralizing antibody response in mice, we generated MS2-VLP-SAP and immunized mice. Serum from these mice showed strong reactivity to the SAP-epitope by ELISA, and we are currently testing them for neutralizing activity. This work identified a novel DENV vaccine target and shows the feasibility of using the MS2-VLP affinity selection technology to perform epitope mapping of the DENV E.

130

SPATIAL ANALYSIS OF DETERMINANTS OF DENGUE TRANSMISSION WITHIN A PROSPECTIVE COHORT STUDY IN VENEZUELA

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Control of dengue and of its mosquito vector has proven challenging in settings of uncontrolled urban growth and unreliable water supply. The ability to identify high-risk areas of dengue transmission can be used to target surveillance and control measures to those locations in a cost-effective manner, particularly in countries where resources are scarce. Despite control measures, transmission of dengue in Venezuela has become perennial with three large epidemics in the past decade. Previous studies in Venezuela using reported epidemiological data show that certain areas are more prone to maintain higher dengue transmission and for longer periods than other. Mapping technology and spatial analysis of epidemiological data will be used to draw risk-maps at a finer scale and identify key factors that determine clusters of high dengue transmission and the spatial spread of dengue within a prospective cohort in Maracay, an endemic city of Venezuela. 2014 individuals aged 5-30 years in 840 households were enrolled between August-December 2010 into a cohort study. Geolocation of households, water bodies and other environmental factors as well as epidemiological data comprising demographic, socioeconomic, clinical, serological and hematological data were collected at baseline. A seroprevalence of 77% was determined at baseline with an estimated 10% of recent infections. Annual cross-sectional surveys determine seroconversion and collect further epidemiological information. Active and passive surveillance is performed to identify dengue cases. Collected data has been imported into geographic information systems software for spatial statistical analysis (regression models) at household level. Risk maps of dengue occurrence measured as confirmed cases by RT-PCR and/or serology will be presented. Implications for dengue control will be discussed.

131

CLINICAL AND HEMATOLOGICAL PARAMETERS ASSOCIATED WITH THE DEVELOPMENT OF DENGUE SEVERE DISEASE IN VENEZUELA

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Dengue is the most important vector-borne viral disease worldwide, with an estimated 390 million infections per year. Dengue can be asymptomatic or present a wide range of manifestations from mild Dengue Fever (DF) to more severe Dengue Hemorrhagic Fever (DHF) and Dengue shock syndrome (DSS). To date, there are no vaccines or antiviral treatments for dengue. At the early stage of the disease, general signs and symptoms of dengue can be confused with others febrile illness (OFI) and, a late dengue diagnosis can be fatal. Dengue in Maracay, Venezuela, is hyper-endemic with co-circulation of the 4 serotypes. In this setting, a longitudinal observational study was set up in 2010 to collect clinical and laboratory parameters of dengue patients. Patients, of all ages, presenting with fever and dengue clinical criteria were recruited from 3 designated health centers, Dengue infection was confirmed by IgM ELISA and/or RT-PCR. Patients were followed daily with clinical examination and sequential blood sampling at determined intervals up to 30 days. Severe cases were treated in a tertiary hospital and followed daily until discharge. Hematological parameters and serum levels of selected biochemical markers were determined in acute phase blood samples. Between August 2010 and January 2013, 206 individuals met the inclusion criteria of which 49% were positive for dengue. Positive individuals were younger than negatives. 20% of positive patients developed alarm signs, while only 7% developed severe dengue. All four serotypes were detected in patients, with DENV-3 predominating. DENV-2 was highly associated with severe dengue cases. Preliminary results show an association of dengue and the presence of exanthema in the first 4 days of the disease. We will present the association of clinical and hematological parameters and the rate of change of the latter with dengue infection, disease severity and parameters discussed above.

132

THE DYNAMICS OF DENV CIRCULATION IN A CITY IN BRAZIL SHOWS A COMPLEX PATTERN OF SEROTYPES AND STRAINS CO-CIRCULATION

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Dengue viruses are members of the genus Flavivirus in the family Flaviviridae and cover 4 antigenically distinct serotypes (DENV-1-4). Although they are nearly identical clinical manifestations, the 4 DENV serotypes are genetically quite distinct. In the present work we looked the Dengue viruses transmission in Sao Jose do Rio Preto (a 400K habitants city in Sao Paulo state, Brazil) from 2011 to 2013. We used serum samples of suspected and confirmed DENV patients provided by the Public Health Authority to profile DENV circulation. The viral surveillance was performed with Multiplex RT-PCR using Flavivirus generic primers based on non-structural protein (NS5), followed by Nested assays with species-specific primers. There were 997 cases confirmed in SJRP from January 2011 to March 2013. We amplified 783 samples for DENV and 327 (41,5%) were positive for DENV-1, 78 (10%) DENV-2, 375 (48%) DENV-4 and 3 (0,5%) DENV-1/DENV-4 coinfection, showing a complex pattern of serotypes circulation. Up to now, 14 DENV-1, 15 DENV-2 and 28 DENV-4 have been subjected to sequencing of the entire envelope gene and were used for phylogenetic reconstruction. The three phylogenetic analyses of serotypes 1, 2 and 4 show that the samples identified in this study grouped with genotypes that circulating in Brazil (genotypes V, Asian American and American, for types 1, 2 and 4 respectively). Looking inside the genotypes two distinct clades formed in DENV-1 and 4 phylogenetic reconstructions, indicating two possible lineages. For DENV-2, one clade grouped all SJRP samples. This data shows that the phylodinamics of dengue circulation can be much more complex than expected even in a small city, with circulation not only of different serotypes but also different strains. These data provide us more information about the dynamics of DENV circulation and its role in emergence of outbreaks and endemic circulation.

133

ENHANCED SURVEILLANCE FOR FATAL DENGUE IN PUERTO RICO 2010-2012

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In Puerto Rico, fatal dengue cases are thought to be under-recognized. To estimate the dengue death rate, an enhanced fatal case surveillance system was implemented in 2010 by CDC Dengue Branch, Institute of Forensic Sciences of Puerto Rico (IFSPR), and CDC Infectious Diseases Pathology Branch. Deaths with a dengue-like, acute febrile illness were identified via 1) disease surveillance, 2) death certificate review, 3) autopsies, and 4) calls to hospitals. Serum was tested by RT-PCR and immunodiagnostic methods for dengue virus (DENV); tissue was tested using immunohistochemistry and RT-PCR. Medical records from dengue laboratory-positive (DLP) case-patients were reviewed. From 2010-2012, 248 suspect dengue fatal cases were identified; most (66%) had autopsy tissues available. Fifty-six cases were dengue DLP, 149 were laboratory-negative, 35 laboratory-indeterminate (acute sample negative and no convalescent sample), and 8 had no specimen; the 2010 incidence rate was 0.1 dengue deaths per 10,000 residents and case-fatality rate was 28 DLP deaths per 10,000 surveillance reported DLP cases. The majority (61%) of DLP case-patients were female and the median age was 45 years (range: 6 months to 89 years) in contrast to only 42% of non-fatal surveillance reported DLP cases being female, with a median age of 18 years. Most case-patients (77%, 43) had one or more chronic medical conditions including 24 with hypertension, 20 with diabetes mellitus type II, and 12 with asthma. Forty (71%) DLP case-patients were admitted to a hospital; 13 died in ER before admission and 3 died at home. Management issues identified included incorrect type of IV fluid usage (14, 25%) and fluid overload (16, 29%). Of 53 case-patients who died in a healthcare facility, 25 (47%) died at night or a weekend. Dengue was listed on the death certificate in only 25 of 54 DLP cases with a death certificate. Our findings suggest that the sex and age distribution of DLP fatal cases differs from DLP cases reported to dengue surveillance. Reasons for these differences are being investigated.

134

ROBUST ACTIVATION AND PROGRESSION FROM ACTIVATION TO TERMINAL DIFFERENTIATION OF NK CELLS DURING ACUTE DENGUE VIRAL INFECTION

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The clinical manifestations of patients with dengue viral infection vary from asymptomatic to dengue fever (DF) and the severe forms of dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS). It is reasoned that innate immune mechanisms particularly during acute infection might play a critical role in the clinical outcome of dengue virus infection. In this study, 39 dengue confirmed patients were recruited. The blood samples were used for flow cytometric analysis of NK cells and their subsets based on the expression of CD3, CD7, CD14, CD16, CD19, CD45, CD56, CD57, CD69 and HLA-DR. The NK cell population was identified as the cells that expressed CD45+/CD14-/CD3-/CD19-/CD7+. The results showed 2 classical NK cell subsets which include the cells that express relatively high levels of CD56 and low to absent CD16 (CD56hiCD16-) and cells that express low levels of CD56 with readily detectable levels of CD16 (CD56loCD16+). As expected, the CD56loCD16+ NK cells comprised the highest frequency and absolute number. A direct correlation was shown to exist between the absolute number of total NK cells and/or the CD56hiCD16- NK cell subset and the day of fever. Furthermore, activated and terminally differentiated NK cells were defined by their expression of CD69 and CD57, respectively. The results showed that both CD69 and CD57 expressing cells were predominantly localized within the CD56loCD16+ population. While acute early disease was characterized by CD69+/CD57- expression by the CD56loCD16+ subset, late acute disease was characterized by CD69-/CD57+ cells within this same subset. The results obtained in this study demonstrate that robust activation during early acute infection of this cytolytic CD56loCD16+ NK cell subset is followed by terminal differentiation of this subset during acute dengue infection and suggested that the kinetics of the changes in the ratio between CD69 and CD57 expressing CD56loCD16+ NK cell subset may play an important role in either providing immunological protection or induction of disease severity. The role of KIR/MHC polymorphisms in regulating the function of these cells is currently under study and may provide novel insights on the severity of dengue infection.

135

PODOCONIOSIS-RELATED STIGMA IN WOLAITA ZONE, SOUTHERN ETHIOPIA: A CROSS-SECTIONAL STUDY

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Studies have indicated that social stigma related to podoconiosis (endemic non-filarial elephantiasis) has a major impact on the psychosocial wellbeing of patients. However, little is known about the extent of stigmatization in various domains of life. We used a recently developed podoconiosis stigma assessment scale to determine the level of felt and enacted stigma as recalled over the previous 12 months. Data collection has been held with 150 patients with podoconiosis and 500 unaffected community members in May 2011. Higher level of stigma has been observed in the ratings of both affected and unaffected persons on felt and enacted stigma scales. The mean scores in the ratings of patients were 19.5 and 21.2 for the felt and enacted stigma scales respectively. 'Major life areas', and 'community, social and civic life' are domains in which enacted stigma was greatest, while most incidences of felt stigma were found at the interpersonal level. The ratings of unaffected people also resulted the mean score of 27.6 for the felt stigma and 17.3 for enacted stigma. Interestingly, there was a statistically significant association between levels of stigma and stage of podoconiosis where stage three patients reported higher levels of enacted stigma (p 0.004). Marginally significant association was observed between felt stigma and stage of the disease (p 0.068). Moreover, the experience of stigma in various domains of life increased with increase in the stage of the disease: enacted stigma sub-score in community and civic life (p 0.003), in major life areas (p 0.017), in interpersonal interaction (p 0.025) and felt stigma sub-score in community and civic life (p 0.021). Specifically, attempt to kill self (48.7%), changing place of residence (49.3%), avoiding visiting of public places (49.3%), fearing to marry unaffected person (42%) are areas where patients with podoconiosis face higher felt stigma. Large proportion of patients also experienced enacted stigma in the form of insults about the foot (52%), mistreatment at workplace (49.3%), being less visited by friends (55.3%) and isolation at social events (44.7%).

Consistent psychosocial support and economic empowerment might help patients reduce internalized stigma while curbing misconceptions and stigmatizing attitudes of unaffected community members towards patients is highly recommended.

136

EQUITY IN UPTAKE OF DIARRHEA AND PNEUMONIA TREATMENT IN A COMMUNITY CASE MANAGEMENT PROGRAM IN UGANDA

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Diarrhea and pneumonia are among the deadliest child killers in poor countries. Integrated pneumonia and diarrhea management was added to community case management of fever to form the integrated community case management (iCCM). Although iCCM aims to improve equity in access to appropriate treatment (AT) using community health workers (CHWs), there is paucity of data on equity in uptake of the new recommended AT for pneumonia (amoxicillin) and diarrhea (oral rehydration therapy plus zinc) in iCCM programs. A before and after study design was used to evaluate equity in uptake of AT among children aged 2-59 months in 2009 and 2012 in nine districts in mid-western Uganda. Data are drawn from random samples of 768 households with 1226 children at baseline survey and 954 households with 1492 children at endline. Concentration indices (C) for equity in uptake of amoxicillin and zinc+ORS were measured across wealth groups. Chi-square tests and logistic regression were used to evaluate the change in AT. Children with symptoms suggestive of pneumonia within two weeks of interview increased from 22% to 31% (p<0.001). There was no change in pneumonia prevalence among the poor at baseline (C= -0.026; 95% CI -0.085, 0.032) and endline (Ci= -0.028; 95% CI -0.067, 0.011). Overall AT for pneumonia with amoxicillin increased from 19% to 41% (p<0.001). There were inequities in AT for pneumonia to the advantage of the less poor both at baseline (Ci= 0.115; 95% CI 0.022, 0.252) and endline (C= 0.009; 95% CI 0.049, 0.068). The odds of receiving amoxicillin for pneumonia were 3 times higher for CHWs compared to other sources (adj. OR= 3.4, p<0.001). No difference in prevalence of diarrhea at baseline (15%) and endline (16%) was observed. However it changed from being less concentrated among the poor at baseline (C=0.021; CI 0.058, 0.099) to high at endline (C= -0.044; CI 0.103, 0.0147). There was no change in diarrhea cases receiving ORS before and after iCCM (41% vs. 46%; P=0.072), however cases of diarrhea receiving zinc+ORS increased significantly from 2% to 27% (p<0.001). The increase in AT for diarrhea seems to favor the poor (C= -0.109; CI -0.226, 0.009) at endline. CHWs were 6 times more likely to treat diarrhoea with ORS+zinc versus other providers (adj OR=6.2, P<0.001). It is evident that the uptake of amoxicillin and zinc improved but sub-optimally in this iCCM program. More advocacy is needed to improve overall uptake of AT especially for diarrhea which is low.

137

MALNUTRITION AND ITS CORRELATES AMONG RURAL PRIMARY SCHOOL CHILDREN AT FOGERA DISTRICT, ETHIOPIA

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Malnutrition is a major public health concern affecting a significant number of school children influencing their health, growth and development, and school academic performance. This study was undertaken to determine the nutritional status of school children in terms of stunting, underweight and thinness and to identify its correlates at Fogera woreda, Northwest Ethiopia, Institutional and community based cross sectional study was conducted from June to December, 2012. The study included 790 primary school children who were selected from the source population by multi stage random sampling technique. Data were collected through interview with parents with a standardized and pretested questionnaire, microscope, physical examination and anthropometric measuring and data were entered and analyzed using SPSS version 16.0 and AnthroPlus softwares. Binary and Multivariate logistic regression analyses were used to identify factors associated with malnutrition among school children. Prevalence of malnutrition was high among school children aged six to fourteen years old (mean age 11.4 ± 2.1 years); Study contents include questionnaire surveys, anthropometric measurement, observation and laboratory methods. Finally 790 school-age students took part in study. The results showed that the overall prevalence of stunting was stunting, underweight and thinness were 243 (30.7%), 96(59.7%) and 294 (37.2%). Those children who were found to be both stunted and underweight were only 1.01% (8). Rice consumption, family size, Family radio, infection, vaccination, latrine availability were significantly associated with malnutrition. However, statistically significant association was not found between malnutrition and parasitic infection and other health conditions. In conclusion, the study found high prevalence of malnutrition (stunting, thinness and underweight). Vaccination, family planning, latrine construction and utilization, rice production and prevention and early treatment of infection were identified as essential interventions to reduce the risk of malnutrition. Ownership of radio should be promoted to reduce malnutrition. However, parasitic infection among primary school children was not significantly associated with malnutrition. But, school children should be targeted to school children.

138

DEVELOPMENT AND PSYCHOMETRIC EVALUATION OF AN INFORMED CONSENT COMPREHENSION QUESTIONNAIRE IN RURAL AND URBAN GAMBIAN RESEARCH POPULATIONS

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Comprehension of study information given during informed consent process remains a major challenge among research participants in low literacy communities of Africa. Written translation and back-translation of informed consent documents pose greater challenges in Gambia because local languages do not have acceptable methods of writing. Furthermore, no adequate methods exist to measure comprehension of study information in this population. We developed a 34-item informed consent comprehension questionnaire consisting of close-ended, open-ended and multiple-choice question items to assess comprehension of key concepts of informed consent information including randomisation, blinding, placebo, therapeutic misconception. The questionnaire underwent face and content validation by a panel of experienced researchers. To overcome the challenge of written translation and back-translation, we audio-recorded the questionnaire in 3 major Gambian languages: Mandinka, Wolof and Fula. The audio-recorded questionnaire was further developed into Audio Computer Assisted Interview format. The formatted questionnaire was administered to 250 clinical trial participants in urban and rural Gambia. The questionnaire was re-administered to half of the participants for a re-test reliability one week after first administration. Principal component analysis showed that most of the question items have strong factor loadings. The questionnaire has high internal consistency with Cronbach's alpha of 0.80 and intra class correlation coefficient of 0.83.

Hypotheses testing also showed the questionnaire has good construct validity. In conclusion, We have developed a reliable and valid measure of comprehension of informed consent information for Gambian research population. This is a major step towards engendering comprehension of consent information among participants with low literacy.

139

CHOLERA TRANSMISSION DYNAMIC MODELS FOR PUBLIC HEALTH PRACTITIONERS

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Great progress has been made in mathematical models of cholera transmission dynamics in recent years. However, little impact, if any, has been made by models upon public health decision-making and day-to-day routine of epidemiologists. This paper provides a brief introduction to the basics of ordinary differential equation models of cholera transmission dynamics. I discuss a basic model adapted from Codeço (2001), and how it can be modified to test different hypotheses, including the importance of asymptomatic or inapparent infections, and hyperinfectious *Vibrio cholerae* and human-to-human transmission. I highlight three important challenges of cholera models: (1) model misspecification and parameter uncertainty, (2) modeling the impact of water, sanitation and hygiene interventions and (3) model structure. I use published models, especially those related to the 2010 Haitian outbreak as examples. I emphasize that the choice of models should be dictated by the research questions in mind. More collaboration is needed between policy-makers, epidemiologists and modelers in public health.

140

TOOLS TO PRIORITIZE COUNTRIES FOR MASS DRUG ADMINISTRATION INTERVENTIONS: A CASE STUDY OF AZITHROMYCIN FOR REDUCING CHILD MORTALITY

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Development assistance for health has undergone a huge expansion in funding over the past decade but funds are not necessarily distributed to countries or communities with the greatest need. As resources for effective interventions become available, the means by which they are allocated among countries is subject to several factors such as a country's income levels, disease burden, and ability or willingness to match funds. However, the relative importance of such factors may be unclear, leading to uncertainty about why some countries are prioritized over others We used promising reductions in childhood mortality reported in a trial of mass drug administration (MDA) of azithromycin (AZM) as the impetus to develop data-driven tools that prioritized countries for potential implementation of AZM MDA programs. We incorporated two considerations: the *opportunity* to reduce mortality and the *feasibility* of implementing such a program, creating *Opportunity* and *Feasibility Indices*, respectively. We limited our analysis to countries with high childhood mortality or morbidity from diarrhea and pneumonia. A *Country Ranking Index* combined key variables from the previous two Indices and applied a scoring system to identify high-priority countries. The Opportunity Index revealed substantial variation in the opportunity for an MDA of AZM program to reduce mortality, even among countries with high overall childhood mortality. The Feasibility Index reinforced the assumption that implementing such a program would be most challenging in the countries that could see greatest benefit. Angola and the Democratic Republic of the Congo received the highest scores in the Country Ranking Index. These visually accessible tools can be adapted for other programs or refined to include other metrics deemed important by stakeholders. The need to explicitly state the metrics and their weighting encourages thoughtful and transparent decision making. This objective and data-driven approach could improve program effectiveness and impact, as well as foster efficient use of resources.

141

INTRODUCING MOBILE PHONE FOR INTERVIEW IN SURVEILLANCE SYSTEM IN BANGLADESH: VALIDATION OF THE METHOD

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Bangladesh while experiencing an epidemiological transition from communicable diseases to non-communicable disease is actually bearing the double burden. The reason why the country is paying equal attention to control both groups of disease. Now a days with increasing complexities of life data collection in person is getting difficult and expensive. Therefore Institute of Epidemiology, Disease Control and Research (IEDCR), Bangladesh decided to introduce mobile phone to conduct interview in surveillance system for communicable (CD) and non-communicable disease (NCD) for the first time in Bangladesh and validate the process. We conducted mobile phone interview (MPI) on 3378 adults in Dhaka city corporation area, using randomly selected phone numbers out of 20,000 numbers provided by an operator. To validate the process we conducted face to face interview (FTFI) on a subset of respondents (401) using same questionnaire. Total 20,916 phone calls were made with a good response rate (61 %). Findings of MPI reflected a specific segment of population using mobile phone who were male (77%), young (90% of respondents were aged 18-44 years), either employed or students (81%), having bachelor degree and above (35%). We compared indicators from both methods and observed similar findings with most (e.g. fever 2 days: MPI 1.3% and FTFI 1.2%, smoker: MPI 33% and FTFI 34 %, Children suffering diarrhea MPI 10% and FTFI 11%) whereas variations with few (e.g. physical activity: MPI 42% and FTFI 66%). Female respondents were less for FTFI (14%) whereas in MPI it is 23%. Afterwards we did sensitivity and specificity test of methods considering FTFI as gold standard and observed findings were good with most of the indicators (e.g. smoker: sensitivity 93%, specificity 95%, Diabetes: sensitivity 83%, specificity 98%) and variation with few (boiling drinking water: sensitivity 92%, specificity 79%, doing physical activity sensitivity 54%, specificity 77%). MPI is convenient, cheap with acceptable sensitivity and specificity in Bangladeshi population. Not getting permission to use Computer Assisted Telephone Interviewing (CATI) software as voice recording is not allowed and noninvolvement of all operators were challenges. IEDCR will scale up mobile phone surveillance system nationwide, taking care of indicators with low sensitivity/specificity, addressing all strata (women, elderly and low socio-economic group) and involving all operators.

142

ASSESSMENT OF *PLASMODIUM FALCIPARUM* AND *TOXOPLASMA GONDII* CO-INFECTION ON PREGNANCY OUTCOMES AMONG WOMEN IN ACCRA-GHANA

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Malaria and congenital toxoplasmosis have been individually reported to cause severe negative outcomes in pregnancies. However, the study on the impact of co-infection of these diseases on birth outcomes is not known. This study investigated the impact of *Plasmodium* spp and/or *Toxoplasma gondii* infections on birth outcomes in women who delivered

at the labour wards of Korle Bu Teaching Hospital. Maternal and cord blood samples were collected into labeled 6 ml tubes after expulsion of the placenta and, placental tissue samples into 15 ml Falcon tubes containing physiological saline. Samples were transported in a cool box to the laboratory for processing. DNA was extracted from portions of the placenta and whole blood using a commercial kit and the remaining span for plasma. DNA was amplified by Nested PCR and products ran on agarose gelto detect *Plasmodium* spp and *T. gondii* in the maternal, cord blood and placental samples using the appropriate primers. Anti *T. gondii* IgG antibodies were detected from plasma using commercial ELISA kits. Demographic data and medical history of participants were obtained from hospital folders. Differences in demographic and obstetric characteristics by co-infection status were assessed by $\chi 2$ (CI=95%, $p<0.05$) to determine the effect of *P. falciparum* and/or *T. gondii* co-infection on pregnancy outcomes. 79 women at delivery aged 18-42 years (mean: 28 years) were in the study and 37.9% (30/79) were multigravids.. The sero-prevalence of anti-*T. gondii* IgG antibodies in maternal and cord blood were 87.9% (60/69) and 51.3% (40/79) respectively. The overall prevalence of *T. gondii* and *P. falciparum* co-infectionwas 15.1% (12/79). There were no statistically significant associations between *T. gondii* and *P. falciparum* infectivity status with birth complications such as still births, birth weights of babies and blood pressure readings of mothers ($p > 0.05$). In this study *P. falciparum* and *T. gondii* co-infections did not aggravate complications associated with pregnancies and deliveries.

143

DEVELOPMENT OF A SCALE TO MEASURE STIGMA RELATED TO PODOCONIOSIS IN SOUTHERN ETHIOPIA

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Health-related stigma adds to the physical and economic burdens experienced by people suffering from neglected tropical diseases (NTDs). Previous research into the NTD podoconiosis showed significant stigma towards those with the disease, yet no formal instrument exists by which to assess stigma or interventions to reduce stigma. We aimed to develop, pilot and validate scales to measure the extent of stigma towards podoconiosis among patients and in podoconiosis-endemic communities. Indicators of stigma were drawn from existing qualitative podoconiosis research and a literature review on measuring leprosy stigma. These were then formulated into items for questioning and evaluated through a Delphi process in which irrelevant items were discounted. The final items formed four scales measuring two distinct forms of stigma (felt stigma and enacted stigma) for those with podoconiosis and those without the disease. The scales were formatted as two questionnaires, one for podoconiosis patients and one for unaffected community members. 150 podoconiosis patients and 500 unaffected community members from Wolaita zone, Southern Ethiopia were selected through multistage random sampling to complete the questionnaires which were interview-administered. The scales were evaluated through reliability assessment, content and construct validity analysis of the items, factor analysis and internal consistency analysis. All scales had Cronbach's alpha over 0.7, indicating good consistency. The content and construct validity of the scales were satisfactory with modest correlation between items. There was significant correlation between the felt and enacted stigma scales among patients (Spearman's $r = 0.892$; $p < 0.001$) and within the community (Spearman's $r = 0.794$; $p < 0.001$). In conclusion,we report the development and testing of the first standardised measures of podoconiosis stigma. Although further research is needed to validate the scales in other contexts, we anticipate they will be useful *in situ*ational analysis and in designing, monitoring and evaluating interventions. The scales will enable an evidence-based approach to mitigating stigma which will enable implementation of more effective disease control and help break the cycle of poverty and NTDs.

144

RISK FACTORS IN THE DOUBLE BURDEN OF MALNUTRITION AND CARDIO-METABOLIC DURING AN ONGOING EPIDEMIC IN BURKINA FASO (WEST AFRICA)

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This study was undertaken to document the double burden of malnutrition and cardio-metabolic risk factors (CMRF) in adults and its occurrence according to different sociodemographic parameters. The design of the study was a population-based cross-sectional observational study. We first randomly selected 330 households stratified by tertile of the income levels proxy in low, middle and high group. Northern district of Ouagadougou, the capital city of Burkina Faso. In each income stratum, 110 individuals aged 25-60y and who had lived permanently in Ouagadougou for at least six months were randomly selected, followed with collection of anthropometric, socioeconomic and clinical data, and blood samples. The overall obesity/overweight prevalence was 24.2% and it was twice as high in women as in men (34.1% vs. 15.5% $p<0.001$). Hypertension, hyperglycaemia and low HDL prevalences were 21.9%, 22.3% and 30.0%, respectively, without gender difference. The prevalence of the metabolic syndrome (MetS) was 10.3%. Iron depletion and vitamin A deficiency affected 15.7% and 25.7%, of subjects respectively with higher rates in women. Coexistence of at least one nutritional deficiency and one CMRF was observed in 23.5% of subjects, and "this double burden" was significantly higher in women than in men (30.4% vs. 16.1%; $p=0.008$), and in the lower income group. In conclusion, CMRF are becoming a leading nutritional problem in adults of Ouagadougou, while nutritional deficiencies persist. The double nutritional burden exacerbates health inequities and calls for action addressing both malnutrition and nutrition-related chronic diseases.

145

ORGANIZATION OF THE HEALTH SYSTEM RESPONSE TO THE 2009 H1N1 INFLUENZA PANDEMIC IN A HOSPITAL IN LIMA, PERU

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Response is especially challenging for hospitals in developing countries with limited resources and crowding due to lack of space. During the 2009 H1N1 influenza pandemic, Hospital Nacional Cayetano Heredia was a reference center for patients for the north of Lima, Peru. Joint meetings with the directors and health specialists in the involved areas of the hospital were held to organize activities, including epidemiologic surveillance, service organization, health personnel training, supplies and materials provision, and biosafety measures. Three progressive stages of triage and care were established in well-ventilated areas, using tents when necessary: 1) Internal medicine and pediatrician specialists at the hospital entrance 2) Infectious disease specialists near the emergency room, and 3) Inpatient unit. Antiviral drugs and personal protection equipment (N95 masks for health care workers and surgical masks for patients) were provided. A case definition was set: fever (38°C) and more than one of the following symptoms: cough, sore throat, rhinorrhea and contact with an infected person. Patients reporting co-morbidities were sent immediately to the second triage stage and hospitalized if necessary. Influenza virus testing was performed only on hospitalized patients. Of the 457 persons meeting the case definition (230 [50%] males, mean age 14 years [SD

18.5], 112 (25%) were laboratory confirmed, 102 (22%) hospitalized, and 9 (2%) died. All fatal cases were detected through the triage system. Co-morbidities in fatal cases were diabetes, obesity, immunosuppression and cardiopathy. Although implemented under emergency conditions with lack of space and healthcare workers required to work extra hours, the response system proved effective. Hospital mortality was lower than in many other hospitals in the world, probably because the triage system allowed us to rapidly identify high-risk groups and provide the necessary attention.

146

ASSESSMENT OF OPERATIONS AND MANAGEMENT OF BARS FOR SAFETY IN UGANDA: A CASE STUDY OF BARS IN RUBAGA AND MAKINDYE DIVISIONS, KAMPALA CAPITAL CITY

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Uganda was ranked as the world's leading consumer of alcohol in the world by WHO in 2004. Bars are the main outlets for alcoholic drinks but seem not to adhere to laws that regulate their operations. In a descriptive survey study, 62 bar operators, each from a bar selected by systematic sampling in Kampala city, Uganda, were interviewed on knowledge about laws governing bars, implementation of such laws, alcohol quality assurance, security precautions, and public health and safety practices in bars. The study was approved by the college research and ethics committee and the city health officer. Data was collected by trained interviewers using a pre-tested questionnaire, entered and analysed using SPSS. About 87 % of bar operators were aware of the existence of the laws regulating alcohol use but could hardly specify them. The law on underage drinking was the most known (62.9%). Some laws are unknown among all bar operators. Exhibiting licenses in conspicuous places was the most broken law (71%). Other laws also commonly defaulted are not respecting opening and closing hours. Most of the bars check on quality of alcohol. Some bars lack security precautions. About 60% of bars had workers trained on hygiene. Only35.5% of the bars had fire extinguishers. There is less supervision of bars by concerned authorities on operation of bars and there is need to sensitize bar operators on the liquor laws and their implementation along with public health and occupational and safety practices.

147

USER FEE POLICY OF MATERNAL AND CHILD HEALTH CARE SERVICES IN BENUE STATE, NIGERIA

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The debate on user fee abound in the literature. It may continue in the foreseeable future as minimal empirical evidence is provided in the current contributions. The bottom line is the ability to pay which rests precariously on the economic status of the households. This is empirically appraised based on the user fee payment for the healthcare of the very vulnerable segment of the society. The user fee payment for the maternal and child health services in Nigeria, the largest concentration of the black race in the world, will give a convincing evidence for the adoption of the health policy in the developing world. In this attempt, the economic status of the rural households particularly their current and medium term assets are analysed using descriptive statistics. The sample size is 275,884 households drawn from Benue State Nigeria. The rural enterprises are also examined using gross margin analytical technique. The user fee is also decomposed all in an effort to examine the ability to pay. The magnitudes of the decomposed user fee comprise Hospital Bed Fee, N 30,176.97 (US $ 188.61) or 33.90%, Charges for Drugs, N 27,426.70 (US $ 171.42) or 30.81% and Informal Fee/Levy of N 18,491.18 (US $ 115.57) or 22.77%. The rural current asset which constitutes the first source for defraying user fee is N 3,895.00 (US $ 24.34). This could hardly offset the first two smallest components of user fee in the rural health care outfit - Registration / Card Fee N 5,026 (US $31.41) and Consultation Fee N 641.61 (US $4.01). The rural medium term asset of N 77,786.00 (US $ 486.16) which is the next source of fund can only liquidate 87.39% of the total user fee. There is a very high, negative, and significant joint movement of rural assets on one hand and many decomposed components of user fee on the other. Thus user fee depletes severely the rural assets. For example, the coefficient of correlation of the rural current asset and charges for drug is -0.81. The Gross Margin which constitutes the major source of replenishment of the household asset is N 93,305.49 (US$ 583.16). This can barely offset the total user fee of N 89,010.96 (US $556.32) leaving a small margin of N 4,294.53 (US $ 26.84) for other competing needs such as shelter, clothing, school fees, etc. The major components of the gross margin are the subsistence animal and crop enterprises, the policy of user fee in a developing economy should therefore be accompanied by policy on the improvement of agricultural productivity.

148

VITAMIN A- AND IRON-RICH FOOD CONSUMPTION BY YOUNG CHILDREN IN A POOR PERI-URBAN COMMUNITY IN THE DOMINICAN REPUBLIC

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Vitamin A and iron are two of the most common micronutrient deficiencies in young children. Nutrition education interventions are one important strategy to address these deficiencies, however, such interventions are often not informed by examination of pre-existing dietary patterns in targeted communities. Such information may allow health message tailoring which may increase impact. The aim of this study was to determine consumption patterns of locally available vitamin A- and iron-rich foods (VAIRFs) by young children in a community targeted for a health education intervention. All caregivers of children under five years of age participating in a community-based growth monitoring service in a poor peri-urban community near Santo Domingo, Dominican Republic, were eligible to participate. At each growth monitoring appointment, child caregivers (n=162) completed structured interviews that included questions on the child's consumption of VAIRFs in the previous seven days. The 421 data points were weighted to one response/child. Eggs and kidney beans represented the most frequently consumed "excellent" sources of vitamin A and iron, respectively. All other sources were reported less than 50% of the time (and less than 30% among children aged 6-12 months). Particularly low consumption values were reported for spinach (13%) and lentils (10%). Preliminary discussions with local stakeholders identified carrot juice and squash as a puree as two candidates for expanded use given regular availability, relative low costs, and palatability for children. Further study is needed to determine factors underlying low consumption patterns and evaluate the extent to which education efforts to expand consumption of VARIFs are successful.

149

THE ASSOCIATION OF KHAT (*CATHA EDULIS)* CHEWING AND ORO-DENTAL HEALTH: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Chewing khat (*Catha edulis),* a plant commonly grown in parts of eastern and southern Africa and southwestern Arabia, has been claimed to be associated with a multitude of oro-dental problems. We searched for available published and grey literatures that reported on the association of khat chewing and outcomes related to oro-dental health using web-based electronic search engines. We used preset inclusion and exclusion criteria for the selection of the identified studies and did a systematic review and

meta-analysis on the selected studies. From the studies obtained through our search, 17 studies were selected for the present review based on our preset inclusion and exclusion criteria. The studies measured different outcomes related to oro-dental health: oral mucousa white lesion, gum recession, periodontal pocketing, gem bleeding, outcomes related to effect of khat on oral microorganisms, and other oral health related outcomes. On the former four categories of outcomes, we performed meta-analysis and the summary effect sizes indicated that khat chewing increases the odds of the respective outcomes. Qualitative synthesis of the findings on the effect of khat chewing on oral microorganisms showed that there is no evidence to claim that khat favours the presence of pathogenic microorganisms in the oral cavity. It rather seems to favour the proliferation microorganisms compatible with oro-dental health. We concluded that khat chewing is associated with adverse oro-dental health outcomes. While there is still a need for generation of more evidences from different countries as there is scarcity of literature in the area, based on the evidence accumulated to date, khat chewing should be considered a threat to oro-dental health and its use be discouraged.

150

OWNERSHIP AND ACCESS TO MOBILE PHONES IN RURAL MALAWI: A NEW CHANNEL FOR COMMUNICATING HEALTH MESSAGES?

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Mobile phone access in sub-Saharan Africa has expanded rapidly in recent years, but dissemination of health information via mobile phones is limited. To explore the feasibility of using mobile phones to communicate health information, we conducted a household census, as part of a larger malaria study, in six rural villages in Machinga District, Malawi in 2012. Residents were asked about access to and household ownership of mobile phones, factors related to ownership and their extent of text messaging. We censused 2657 households, of which 2430 (91.5%) had an eligible respondent who consented to participate. Respondents were mostly female (76%) and had a median age of 33 years. Of the households surveyed, 42% owned at least one mobile phone and 4% owned more than one phone. In households without a phone, a majority (59%) knew a neighbor with a phone and almost half (46%) received personal messages from the neighbor's phone. Most respondents from households with phones had attended primary school or above (73%), could sign their own name (70%) and many could correctly read a short text message in Chichewa, Chiyao, or English (53%). Household mobile phones were used for sending (39%), receiving (69%) and reading (46%) text messages, which contained personal messages and advertisements. Only 7% of households received health information text messages in the week prior to the interview. Mobile phone ownership was significantly associated with ability to read a text message (OR 2.2; 95% CI 1.8-2.6), increased household size (OR 1.3; 95% CI 1.2-1.4), bednet ownership (OR 2.1; 95% CI 1.6-2.8) and was negatively associated with having children under the age of five years (OR 0.6; 95% CI 0.5-0.7). Household mobile phone ownership is approaching the same level of coverage as radios (55%) in our study area and phones should be explored as a communication route for behavior change messages. Additional studies are needed to compare sociodemographic factors related to ownership of mobile phones and to determine whether text or voice messages would be the most effective method for disseminating information through mobile phones in rural populations.

151

EFFECT OF MALARIA-INDUCED HEME/HO-1 ON PREGNANCY OUTCOMES

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Plasmodium falciparum malaria threatens about 200 million people worldwide resulting in 655,000-1000000 deaths annually with pregnant women and children at high risk. Malaria in pregnancy causes severe maternal anemia, low birth weight deliveries and maternal and infant mortality. Although current anti-malarial treatment are effective in targeting parasites, recent studies have shown that the pathogenesis of severe malaria is not only due to *parasitemia* but also by parasite derived factors and host factors such as heme and heme oxgenase-1 (HO-1) as a result of hemolysis. Furthermore we have shown that heme and HO-1 are involved in the pathogenesis of experimental cerebral malaria. In this current study we determine the effect of malaria-induced heme and HO-1 on pregnancy outcomes. We hypothesized that pregnant women with placental malaria will have high levels of Heme/HO-1 and poor pregnancy outcomes than pregnant women without malaria. We measured the Heme and HO-1 levels in plasma samples from pregnant women with and without malaria and correlate it with their pregnancy outcomes. The preliminary results showed that pregnant women with malaria had significant higher mean levels of heme (80.160.4) than pregnant women without malaria (66.131.6), p = 0.006. Malaria (+) pregnant women had significant higher median HO-1 levels (5.8,10.1) than Malaria (-) pregnant women (3.3 ,7.9), p = <0.001. The assessment of HO-1 polymorphisms are currently being performed and the results will be discussed during the symposium. In conclusion, malaria in pregnancy is associated with increased Heme and HO-1 reflecting the degree of hemolysis induced by parasites (sequestered or systemic) and pregnancy outcomes. Findings from this study may provide insight in effect of malaria derived heme and HO-1 in pregnancy which may result in development of preventive chemotherapy that target both parasites and hemolysis or reduce the levels of heme in pregnancy during malaria infection.

152

THE ROLE OF TAU PROTEIN IN THE NEUROPATHOGENESIS OF CEREBRAL MALARIA

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Cerebral malaria (CM) is a potentially fatal neurological manifestation of disease primarily caused by infection with *Plasmodium falciparum*. Despite effective anti-malarial therapy, approximately 20% of CM survivors develop long-term cognitive and behavioral deficits, however the mechanisms that mediate this neurological impairment are not well understood. Neuronal injury has been associated with the neurocognitive deficits in several neurodegenerative diseases and may contribute to the impairment seen in CM. In this regard, damage to neuronal axons has been observed in both human and murine experimental CM (ECM). Furthermore, improper regulation of tau protein, an axonal protein important for microtubule stability and cytoskeletal organization, has been demonstrated in mouse and human disease. We hypothesized that the neuronal injury observed in ECM results, in part, from abnormalities in tau. Improper regulation of tau results in an increase in the phosphorylated levels of the protein. We quantified the levels of two specific forms of phosphorylated tau known to be pathological in Alzheimer's disease (Ser396/404; Ser202) in several brain regions of mice with ECM and compared our findings with uninfected mice and mice infected with a less neurotropic malarial strain. In the same brain regions, we also quantified the level of SMI32, a marker of axonal damage. We found that both forms of phosphorylated tau and SMI32 are elevated throughout the brains of mice with neurological disease. We then discovered that treating ECM

mice with the immunotherapeutic paired-helical filament-1 antibody, which clears phosphorylated tau in mouse models of Alzheimer's disease, restores normal tau and reverses axonal damage in certain brain regions, suggesting that this protein is contributing to the neuronal injury in ECM. Our goal is to further establish tau as a significant contributor to the pathogenesis of CM. This protein may prove to be a viable target to ameliorate both the neuronal damage and neurocognitive impairment which occur during disease.

153

MALARIA EPISODES IN MALIAN CHILDREN: IMPACT OF HBS AND HBC

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We conducted a cohort study from 2008 to 2011 in three villages in Mali Sudano-Guinean zone. The study focused on children from 6 months to 17 years who were followed every year beginning at the end of the malaria transmission season. At baseline, we determined the hemoglobin type and level for all children. In total, we enrolled 1559 children over the four year. The sex ratio was 1.01 in favor of boys. Children aged 0-5 years were the majority (51.22%). We observed 4182 cases of malaria during the four malaria transmission seasons. 71.4 % of children had at least one malaria episode. Frequencies of HbS and HbC were respectively 14.5% and 6.5%. The mean hemoglobin was lower in subjects with HbAS (95% CI 8.7-11.4). On enrollment, AS children were more likely to be anemic than normal children (p = 0.0001). The number of malaria episode was significantly reduced for HbAS than that in HbAA and HbAC ($p = 3.10^{-6}$ and 10^{-8}). The mean parasite density/µl for HbAS of 19402.65 (95% CI, 7577.57 - 46382.87) was significantly lower than that of than HbAA 24277.68 (95% CI, 6509.64 - 55 065) (p = 0.002).The mean parasite density varied with age and was highest for children aged 0-5 years ($p=10^{-6}$). In conclusion, the mean parasite and the average number of malaria episodes decreased significantly for HbAS.

154

ROLE OF CYTOKINE-MEDIATED ENDOTHELIAL ACTIVATION PATHWAY IN PATHOGENESIS OF COMPLICATED *PLASMODIUM VIVAX* CLINICAL ISOLATES FROM PAKISTAN

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Plasmodium vivax is the prevalent malaria species contributing 70% of malaria burden in Pakistan. Though considered benign, complicated cases of *P.vivax* are consistently being documented from this region. It has been hypothesized that *P.vivax* utilizes cytokine-mediated endothelial activation pathway as a mechanism to manifest severe disease symptoms. Therefore, we aimed to test this hypothesis by designing a case control study using well-characterized groups of uncomplicated (n=100), complicated cases (n=82) and healthy controls (n=100). Concentrations of cytokines, TNF-α,IL-6, IL-10 and endothelial activation markers ICAM-1 (Intracellular adhesion molecule-1), VCAM-1(Vascular adhesion molecule-1) and E-selectin were determined by Enzyme-Linked immunosorbant assay (ELISA). Correlation of cytokines and endothelial activation markers was done using Pearson two way correlation matrix. Furthermore, the significance of these biomarkers as indicators of disease severity was also analyzed. The results showed that TNF-α, IL-10, ICAM-1and VCAM-1were 3-fold, 3.7 fold and 2 fold increased between uncomplicated and complicated cases while IL-6 and E-selectin was 1.8 and 1.2 fold decreased between the two groups. Comparison of healthy controls with uncomplicated cases showed showed no significant difference in TNF-α concentrations while IL-6,IL-10,ICAM-1,VCAM-1and E-selectin were found to be 3.5-fold,20-fold,3-fold,4-fold and10-fold elevated respectively. Furthermore, significant positive correlation was observed between TNF-α and IL-10, TNF-α and ICAM-1, ICAM-1 and VCAM-1. A Receiver operating curve (ROC) was generated which showed that TNF-α, IL-10, ICAM-1 and VCAM-1 were the best individual predictors of complicated *P.vivax* malaria. Therefore, it is concluded that cytokine-mediated endothelial activation pathway is the possible mechanism of pathogenesis in *P.vivax* and cytokine and endothelial activation markers may serve plausible biomarkers of complicated *P.vivax* infection.

155

INTENSITY OF PARASITE SEQUESTRATION IN RETINAL VESSELS CORRELATES WITH SEVERITY OF RETINOPATHY IN MALAWIAN CHILDREN WITH CEREBRAL MALARIA: A HISTOPATHOLOGICAL STUDY

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Ocular funduscopy to detect malaria retinopathy (MR) affords an opportunity to obtain diagnostic and prognostic information on children diagnosed with cerebral malaria (CM) in endemic countries. We investigated the correlation between sequestration of parasitized red blood cells (pRBC) in the neural retinal vasculature and *pre mortem* imaging, in the context of a unique long-running autopsy study. We performed a case-control study using retinal photography and fluorescein angiography. The cases were children with autopsy-proven CM (n=5) and the controls were parasitemic children with a non-malarial cause of coma (n=3). HIV serostatus was determined by rapid tests (n=5 HIV+, n=3 HIV-). Eyes enucleated *post mortem* were processed for histological evaluation, to determine % vessels parasitized in retina and choroid. Severe retinal whitening, vessel discoloration and perfusion abnormalities were seen in 4 CM cases clinically and on imaging. One case was graded as mild MR, defined by the presence of mildest severity categories for each sign during clinical examination. Histopathology in severe MR cases showed pRBC sequestration in median 85% (min-max: 73-95%) and 95% (min-max: 85-100%) in retinal capillaris and venules respectively. The one case with mild MR showed 19% and 36% respectively, and controls 0% (except one case with 4% retinal venules pRBC positive). pRBC sequestration in choriocapillaris (15%, (0-20%)) and choroidal venules (44%, (14-46%)) was less than in the retina in severe MR, as well as in mild MR (choriocapillaris - 5%, and choroidal venules - 21%). Sequestration of pRBC was not associated with HIV status. Our histopathological evidence to date suggests a correlation between increasing severity of MR and higher density of pRBC sequestration in retinal capillaries and venules. Lower density of pRBC in choroidal vessels indicates differential pRBC sequestration between different ocular tissues, and the role of differential expression of endothelial receptors needs to be investigated.

156

POPULATION WIDE SURVEY OF GENE COPY NUMBER VARIATION IN NATURAL POPULATIONS OF *PLASMODIUM FALCIPARUM*

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Gene copy number variants (CNVs), which consist of deletions, amplification and inversions of single or sets of contiguous genes, contribute to the great diversity in the *Plasmodium falciparum* genome. CNVs may influence the expression of genes and hence may affect important parasite phenotypes such as virulence, drug resistance, persistence and transmissibility. We hypothesize that CNVs may be important for adaptation of the parasite to its highly variable environment. To investigate this hypothesis, we conducted a population wide survey of CNVs in 191 fresh field isolates from three populations in Eastern Africa with different malaria transmission intensities. To detect CNVs, we performed comparative genome hybridization using a 70mer microarray. We have identified approximately 110 CNVs, with some varying significantly in frequency among the populations. We plan to validate our findings by performing whole genome sequencing. Furthermore, we will investigate the influence of CNVs on gene expression by relating CNV data to whole-genome transcription profiles. Overall, we hope to describe the amount of CNV-associated variation in gene expression in *P. falciparum* parasites when in their natural environment.

157

EXPRESSION OF RECOMBINANT *PLASMODIUM VIVAX* DUFFY BINDING PROTEIN REGION II (PVDBPII) AND *P. FALCIPARUM* ERYTHROCYTE BINDING PROTEIN REGION F2 (PFEBA-175F2) TO DETECT IMMUNE RESPONSE TO MALARIA ANTIGENS

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Specific ligand - receptor interactions is essential for malaria parasites to continue their development in human erythrocytes. A 175 kDa *Plasmodium falciparum* erythrocyte-binding antigen (EBA-175) and its *P. vivax* orthologue, the Duffy binding protein (DBP), are vital ligands playing a key role in invasion. Residents living in endemic regions naturally acquired antibodies against these ligands making these molecules attractive as malaria vaccine candidates. However, the development of a broadly effective PfEBA-175F2 and PvDBPII-based vaccine is compromised by the presence of polymorphisms as a result of the action of positive selection by host immune pressure. In this study, we attempted to generate recombinant of PvDBP region II and PfEBA-175 region F2 representing selected alleles found to be commonly circulating in Timika-Papua, Indonesia to provide tools to better understand immune responses to malaria. Recombinant PvDBPII and PfEBA-175F2 proteins were expressed as fusion proteins in *Escherichia coli* with 6xHis tag at the C-terminal end and purified by Ni-NTA metal affinity chromatography under denaturing conditions. We successfully expressed one full-length haplotype of PvDBPII INA21 possessing 14 different amino acid residues compared to the reference strain Sal-1. We tested a small number of sera from *P. vivax*-infected individuals from Timika-Papua to the reduced recombinant PvDBPII-INA21 and PvDBPII-Sal-1 (a gift from Professor Adams) by Western blot assay. Antibodies in most sera from *P. vivax*-infected patients recognized recombinant PvDBPII-INA21 but did not react to PvDBPII-Sal-1 suggesting Sal-1 type may not be commonly circulating in Timika. Positive serological response of some sera tested indicating the presence of immunogenic cryptic linear B-cell epitopes on the native protein. This result supports the development of PvDBPII-based vaccine against *P. vivax* blood-stage. In parallel, recombinant EBA-175 region F2 has also been successfully expressed in this study. Further work is required to produce a large-scale recombinant protein of PvDBPII and EBA-175F2 to allow testing all the sera collected from Indonesia to check for their cross-reactivity to these malaria antigens.

158

HAPTOGLOBIN AND OROSOMUCOID LEVELS IN SUDANESE PATIENTS WITH UNCOMPLICATED AND CEREBRAL MALARIA IN RELATION TO HP POLYMORPHISM

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The levels of haptoglobin (Hp) and orosomucoid (ORO) in 187 noncomplicated malaria patients, 23 cerebral malaria patients and 24 healthy controls were determined according to haptoglobin phenotypes and clinical presentation of malaria using quantitative nephelometry. Hp1-1 levels of Hp showed decline among patients with noncomplicated and cerebral malaria (mean levels were 0.518g/L and 0.62g/L respectively) compared to 1.21g/L in healthy controls). Hp 2-1 and 2-2 showed increased levels among uncomplicated malaria patients and slightly decreased levels among cerebral malaria patients. Orosomucoid levels increased in uncomplicated and cerebral malaria patients with all Hp phenotypes in comparison to healthy control group (0.745 g/L in healthy control, 1.382 g/L in uncomplicated malaria cases and 1.282 g/L in cerebral malaria cases). These results indicated a role for haptoglobin gene polymorphism and ORO plasma levels on the outcome of infection. Possible implications these molecules in malaria infection will be discussed.

159

CORRELATING MICROGLIAL AND ASTROCYTE ACTIVATION WITH CLINICAL AND HISTOLOGICAL EVIDENCE OF DISEASE IN EXPERIMENTAL CEREBRAL MALARIA

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Severe malaria, chiefly cerebral malaria (CM), claims close to 1 million pediatric lives per year. The role of glia in this devastating disease is incompletely elucidated. We hypothesized that glial activation would be positively correlated with intracerebral hemorrhages and *parasitemia* levels, and negatively correlated with a murine behavior score. To investigate the relationships between behavior, intracerebral hemorrhage, *parasitemia*, and cellular dysfunction in a murine model of CM, cohorts of C57BL/6 mice infected with *Plasmodium berghei* ANKA (PbA) and non-infected controls were serially sacrificed on days 2, 4, 6, 8, 9, and 10 of infection, and brain tissues were analyzed. Parasitemia and behavior were assessed daily. We used the rapid murine coma and behavior scale (RMCBS), which ranges from 0 (lowest function/ill) to 20 (highest function/healthy). H&E stained intracerebral hemorrhages were quantified via microscopy in experimental and control mice. The activation of glia in the CA1 region of the hippocampus and MO1 and MO2/3 somatomotor areas of the frontal lobe of experimental and control mice was quantified using immunohistochemical (IHC) markers for glial fibrillary acidic protein (GFAP), calcium binding protein (S100B), and ionized calcium-binding adapter molecule-1 (Iba1). GFAP and S100B reflect astrocyte activation and Iba1 reflects microglial activation. The data revealed a significantly higher microglial cell count in experimental mice relative to controls in both the CA1 ($p < 0.002$) and MO ($p = 0.005$) regions, as evidenced by upregulated Iba1, whereas the upregulation of GFAP and S100B via IHC assessment was inconclusive. Microglial activation was positively correlated

with intracerebral hemorrhage count (CA1: r2 = 0.61, p = 0.04; MO: r2 = 0.62, p = 0.04) and *parasitemia* (CA1: r2 = 0.57, p = 0.06; MO: r2 = 0.74, p = 0.01), and was inversely correlated with behavior score (CA1: r2 = -0.65, p = 0.01; MO: r2 = -0.68, p = 0.01). Overall, microglia appear to be highly activated in the experimental CM model and correlate with known histological and clinical pathology, whereas astrocytes do not appear to be as greatly affected. These results may help investigators better understand the role of glia in CM.

160

THE MICROVASCULAR DISTRIBUTION OF PARASITE SEQUESTRATION IN PEDIATRIC CEREBRAL MALARIA

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The sequestration of parasitized red blood cells (PRBC) has been observed in all three vessel types (arteries, capillaries, and veins) in human cerebral malaria (CM) over the past 100 years, but the relative amounts have not been quantified. The brain vessels of three Malawian children, who satisfied the clinical definition of CM, were examined for PRBC sequestration. Vessel types were identified by structure and staining patterns on immunofluorescent images using 4′,6-diamidino-2-phenylindole (DAPI), fluorescein isothiocyanate (FITC), and Texas Red stains, which labeled DNA, smooth muscle actin, and endothelial cells, respectively. Vessels were documented as parasitized or non-parasitized based on the presence or absence, respectively, of parasitized erythrocytes, as evidenced by morphological features in hematoxylin and eosin stains. Parasite sequestration was reported as a percentage of parasitized vessels in the region of interest. This novel method of analysis revealed the presence of parasites in all three vessel types, with various degrees of sequestration. Parasites were most numerous in venules (96.9 ± 9.6%), followed by capillaries (92.6 ± 12.9%), and then arteries (82.8 ± 35.6%) (p = 0.0057); there were no significant differences in the distribution of parasitized vessels between the different CNS regions examined (frontal lobe, temporal lobe, occipital lobe, hippocampus, caudate nucleus, thalamus, midbrain, pons, medulla, cerebellum and spinal cord). These findings support the hypothesis that the sequestration of parasitized red blood cells may lead to venous obstruction and vascular congestion, which could result in ischemia and increased brain volume. The latter is a phenomenon highly correlated with a fatal outcome in CM.

161

LONG-TERM CONTINUOUS CULTURE SYSTEM FOR *PLASMODIUM VIVAX*

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Plasmodium vivax is considered as the most widely distributed human malaria parasite in Tropical countries and temperate zone. Study on *P. vivax* has much lagged behind mainly due to the unavailable of the *in vitro* continuous culture eventhough some have shown the potential. In this study, the effect of two major factors have been studied. (i) Host reticulocyte. Different sources of host reticulocytes, peripheral blood, cord blood and hematopoietic stem cell derived-reticulocyte, have been comapred. In term of parasite invasion, all three sources of reticulocyte can support parasite invasion in similar fasion but only reticulocytes enriched from peripheral blood showed the better maturation of the parasite. Moreover, the amount of reticulocyte that need to be maintained in the culture has also been optimized. (ii) Culture medium. Different culture mediums have been compared, McCOY'5A, RPMI 1640 and Waymouth. McCOY'5A medium supplemented with 25% heat inactivated human AB serum, which further selected as a standard culture medium for *P. vivax* in our culture system, shown better support maturation of the parasite in long term. In addition, the standard McCOY'5A culture medium has been modified by adding additives which targeting major metabolic pathways of the parasite. This modified medium showed better support for long term parasite culture. With the optimized culture system, fresh isolates *P. vivax* have been maintained for more than 5 months in this *in vitro* culture system

162

IDENTIFICATION OF *PLASMODIUM FALCIPARUM* GENES INVOLVED IN PARASITE ADAPTATION TO HOST IMMUNITY

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Malaria parasites have an extraordinary ability to adapt. This underlies their outstanding success in the face of strong and highly diverse selection pressures in their environment, both natural and man-made. We have used whole-genome transcriptome analyses to test for adaptive differences between natural populations of *Plasmodium falciparum* parasites evolved under different transmission intensities in East Africa. Comparisons from three pairs of parasite populations from high vs. low transmission intensity environments revealed differences in functions relating to red cell invasion, membrane and protein transport, energy metabolism, protein turnover and export to the red cell cytosol. This suggests that parasites evolve different levels of investment in replication and immune evasion to suit their prevailing environment. The molecular systems and genes involved in this adaptation have been identified.

163

ROLE OF PLATELETS AND IMMUNE CELLS IN BLOOD-BRAIN BARRIER FUNCTION DURING EXPOSURE TO *PLASMODIUM FALCIPARUM*-INFECTED RED BLOOD CELLS: ELECTRICAL CELL-BASED STUDY

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Cerebral malaria (CM), a severe neurological complication of *Plasmodium falciparum* infection, is a leading cause of mortality in many regions of the world, and its underlying mechanism and consequences are still not fully understood. Sequestration of *P. falciparum*-infected red blood cells (Pf-IRBC) and specific immune responses are key contributors to the pathological alteration of the blood-brain barrier (BBB) and microvasculature. In the present study, the effects of Pf-IRBC, peripheral blood mononuclear cells (PBMC) and platelets on the function of the BBB have been successfully investigated using electrical cell-substrate sensing (ECIS) in conjunction with immunostaining of tight junction protein. Primary cultures of porcine brain capillary endothelial cells (PBCEC) were implemented as a potential *in vitro* model of the BBB. The exposure of the PBCEC to Pf-IRBC (trophozoite stage with 1% Hematocrit (Hct) and 50% Pf-IRBC), PBMC (7.0×10^5 cells/cm^2) and platelets (1×10^9 cells/cm^2) rapidly decreased the barrier function (the electrical resistance of the cell-cell contact; R_b) and cell-substrate function (the frequency-dependent impedance contribution of cell-substrate adhesion; α) to 80% within 4 h. The immunostaining of tight junction protein confirmed the disruption of Claudin-5 at the site of cell-cell contact. This study presents the first investigation of BBB dysfunction during stimulation with Pf-IRBC, PBMC and platelets. Our findings support the hypothesis that parasitized red blood cells and the host response to these cells together play a role in inducing vascular leakage and the disruption of several vascular beds, particularly in the brain vasculature forming the BBB.

164

HEMOPEXIN AS A POTENTIAL PROTECTIVE FACTOR IN SEVERE MALARIA

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Severe malaria continues to have a high mortality rate despite the use of potent anti-malarials. Host response is an important determinant of disease severity and outcome in severe malaria. Adjunctive therapies targeting host response may further improve outcome over that possible with anti-malarials alone. During malaria infection both infected and uninfected red blood cells undergo hemolysis releasing hemoglobin. Cell free hemoglobin not bound by haptoglobin can generate cytotoxic free heme. Hemopexin is an endogenous protein that binds and transports free heme for degradation. To investigate the role of hemopexin during malarial infection, we measured plasma levels of hemopexin, free heme, haptoglobin and free hemoglobin in a case control study nested within a prospective cohort study of Ugandan children with malaria (severe malarial anemia (SMA), n=27; cerebral malaria (CM) n=31; and uncomplicated malaria (n=29)) and compared the values using a Kruskal-Wallis test followed by Dunn's Multiple Comparison test. Levels of free heme were significantly higher in children with SMA (median [IQR]: 11.1 uM[2.4, 26.9], p<0.01) or CM (15.8[5.2, 30.6], p<0.001) compared to those with uncomplicated malaria (1.0 [0.5, 6.5]). Whereas levels of hemopexin (Hpx) and haptoglobin (Hpt) were significantly higher in patients with uncomplicated malaria (Hpx 993 ug/mL[766, 1144]; Hpt 109.0 ug/mL[6.0, 1130.0]) compared to patients with SMA (Hpx 221[63, 338], p<0.001; Hpt 2.0[2.0, 6.0], p<0.001) and CM (Hpx 292[106, 525], p<0.001; Hpt 7.0[3.0, 17.0], p<0.05). These observations support the hypothesis that hemopexin may play a protective role during malaria infection. Based on this hypothesis, we are investigating the mechanism and causality of the heme axis in the *Plasmodium berghei* ANKA (PbA) model of experimental cerebral malaria and examining outcomes of PbA infection in hemopexin knockout mice with and without supplementation with exogenous hemopexin. These experiments aim to validate the use of the PbA model to evaluate the therapeutic potential of exogenous hemopexin during severe malaria.

165

HIGH LEVELS OF ERYTHROPOIETIN ARE NOT ASSOCIATED WITH NEUROPROTECTION IN UGANDAN CHILDREN WITH CEREBRAL MALARIA

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High plasma levels of erythropoietin (EPO) were associated with protection from acute neurologic deficits in children with cerebral malaria (CM) in one study, but these study findings have not been replicated, and there are no studies to date on the association of plasma EPO levels with long-term neurologic deficits. As a result of these findings, clinical trials of recombinant human EPO (rhuEPO) in children with CM have been initiated. However, clinical trials of rhuEPO in other neurologic conditions have generated conflicting results. We conducted a study in children with CM in Kampala, Uganda to assess the association of plasma and cerebrospinal fluid (CSF) EPO levels with neurological outcomes and mortality. Plasma EPO levels were measured by radioimmunoassay in 177 children with CM, of whom 119 had sufficient CSF for EPO testing. CSF tumor necrosis factor-α (TNF-α) levels have also been associated with neurologic deficits in CM, so plasma and CSF TNF-α levels were measured by cytometric bead assay. Coma duration predicted neurologic deficits at discharge (P<0.001), and a higher number of seizures (P=0.001), worse coma score (P=0.003) and longer duration of coma (P<0.001) all predicted neurological deficits at 6 months. Neither plasma nor CSF EPO levels differed in children with vs. without neurologic deficits at discharge or 6-month follow-up (P>0.4 for all), and risk of neurologic deficit at discharge or 6 months adjusted for age, hemoglobin level, coma score, coma duration and number of seizures did not differ according to CSF or EPO level. Plasma and CSF EPO levels are not associated with short or long-term neuroprotection in Ugandan children. Caution may be warranted in consideration of rhuEPO as adjuvant therapy for neuroprotection in cerebral malaria.

166

EVALUATION OF THE ACUTE TOXICITY OF THE ANTIPLASMODIAL ACTIVE DICHLOROMETHANE/METHANOL EXTRACT FROM *CASSIA ALATA L.* LEAVES

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In Burkina Faso *Cassia alata L.* (leaves) is a partner plant in the formulation of standardized remedy "Saye" used in decoction for the treatment of uncomplicated malaria. The aim of the study was to evaluate the acute toxicity of the antiplasmodial active dichloromethane/methanol extract of the leaves of *Cassia alata L.* in NMRI mice. The acute toxicity test was performed according to the method of Thompson and Weil (1952). Group I served as a control and groups II, III, IV and V received orally increasing doses (823.5, 1235.25, 1853, and 2779.5 mg/kg /body weight respectively. LD_{50} were assessed according to the method of Litchfield Wilcoxon at the higher dose level of 5000, 7500, 11250 and 16875 mg/kg body weight. At this step, male and female mice of 9 weeks of age were used at each dose orally administrated. There were no dead mice of either sex in any group and no changes in clinical signs. So, the maximum non lethal dose was 16875 mg/kg in mice. At high dose up to 5000 mg/kg, there was a significant reduction of body weight gained in treated mice group comparing to control group. But, this reduction of body weight gained was more significant in male mice comparing to female mice. Any histogical change was detected in the different organs. A significant increase of total protein and total cholesterol were obtained with mice receiving the high dose compared to the control showed. Glucose was low in groups of mice which have received 823.5, 1235.25 and 1853 mg/kg bw of plant extract compared to the control group. An increase of urea was seen in mice treated with 1235.25 mg/kg bw while creatinine increased significantly in all treated groups compared to control group. The enzyme value AST was not different in the treated group compared to the control, but ALT was significantly different in the treatment group. White blood cells count showed a significant increased at 823.5 mg/kg bw comparing to control group. In conclusion, no adverse effects were observed in any of the rodents used in the study and no deaths occurred in any of the mice. Some differences were observed in hematological and biochemical parameters but no overt toxicity at the dose levels of the extract tested. The antiplasmodial active dichloromethane/methanol extract from *C. alata* L. Leaves were practically non-toxic.

167

POPULATION PHARMACOKINETICS AND PHARMACODYNAMICS OF ARTEMETHER-LUMEFANTRINE IN PREGNANT AND NON-PREGNANT WOMEN WITH UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN TANZANIA

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Artemether-Lumefantrine (AL) is the first line treatment for uncomplicated malaria in second and third trimester of pregnancy. Its efficacy has recently been challenged in pregnancy due to altered pharmacokinetics (PK) properties in this vulnerable group. The aim of this study was to determine PK profile of AL in pregnant women compared to non-pregnant women, and assess for therapeutic outcome. Thirty-three pregnant women and matched 22 non-pregnant women were treated with AL (80/480 mg) twice daily for 3 days. All patients provided five venous plasma samples for drug quantification at pre-defined random time over 7 days. Inter- and intra-individual variability were assessed and covariates effects quantified using a nonlinear mixed-effect modeling approach (NONMEM®). One-compartment model with first-order absorption and elimination and linear metabolism from the drug to the metabolite fitted the data at best for both arthemether (AM) and lumefantrine (LF) and their metabolites. Pregnancy status and diarrhea showed a significant influence on LF pharmacokinetic. Lumefantrine bioavailability and metabolism rate in pregnant women were respectively 34% lower and 78% higher than in non-pregnant patients. Total therapeutic failure was 7 (13%), 18% pregnant and 5% non-pregnant. A high median day 7 lumefantrine concentration was associated with adequate clinical and parasitological response (1,070 Vs 730) ng/ml. In simulation of the lumefantrine, splitting the same recommended 6 dose of AL over 5 days regimen would greatly reduce the likelihood of exhibiting sub-therapeutic drug concentrations. In conclusion, the observed reduction in lumefantrine bioavailability during pregnancy explains the high therapeutic failure in this group. Hence, modified treatment regimen of malaria in pregnancy should highly be considered. Comparative studies that explore the role of host parasite immunity on therapeutic response during pregnancy are important for consideration.

168

IDENTIFICATION OF 3, 4', 7 TRIHYDROXY-5-METHOXYFLAVONE IN AN ANTIPLASMODIAL FRACTION OF *CHROMOLAENA ODORATA* WITH HIGH ACTIVITY AGAINST CHLOROQUINE RESISTANT *PLASMODIUM FALCIPARUM*

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The evolution and spread of malaria contributes to high mortality rates in many tropical areas of the world. *Plasmodium falciparum* malaria remains the biggest challenge in chemotherapy, as drug treatment options against drug-resistant parasites are limited and an effective protective vaccine is still unavailable. Thus, new antimalarial drugs that are effective against drug-resistant parasite strains are urgently needed. *Chromolaena odorata* is a shrub native to tropical central and South America where preparations of its leaves are used as remedy for malaria fever. This study was undertaken to evaluate the antiplasmodial activity of *C. odorata* extracts in a mouse model of infection. Column fractions obtained from the most active extract were also tested for activity against THP-1 cell line, chloroquine sensitive (HB3) and chloroquine resistant (FCM29 *Plasmodium falciparum*. Furthermore, a marker compound was identified in the most active fraction and characterized using its physicochemical and spectroscopic data (1H-, 13C- NMR and UV spectroscopies). A batch of powdered leaves of *C. odorata* was successively extracted with hexane, dichloromethane, methanol and water. The extracts were subjected to the classical Peters' 4-day suppressive test in mice against *P. berghei*. The dichloromethane extract was most effective; significantly ($P<0.05$) suppressing infection by 99.35 % at 100 mg/kg body weight. This extract was further separated into 13 sub fractions (CO2A - CO2M) by column chromatography using a gradient mobile phase system of hexane, ethylacetate and methanol. Results showed that CO2K was most active, with IC_{50} of 4.8 and 6.74 µg/mL against *P. falciparum* HB3 and FCM29 respectively. The fraction (100 µg/mL) was non cytotoxic against THP-1 and its separation by column chromatography yielded a flavonoid which was characterized as 3, 4', 7 trihydroxy- 5-methoxyflavone. This compound can serve as a useful phytochemical marker of the antiplasmodial active fraction of *C. odorata*, which exhibits potential for development as medicine against malaria.

169

EARLY PARASITE CLEARANCE FOLLOWING TREATMENT WITH ARTEMISININ-BASED COMBINATION THERAPY AMONG UGANDAN CHILDREN WITH UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA

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Artemisinin-based combination therapy (ACT) has become the most widely recommended first-line therapy for *Plasmodium falciparum* malaria worldwide. Artemisinin resistance has now been reported in Southeast Asia with a clinical phenotype manifested by slow parasite clearance evidenced by up to 50% of patients having asexual parasites detected by microscopy after 3 days of artemisinin monotherapy. Although arteimisinin resistance has not been reported in Africa, there is a need to understand the dynamics of parasite clearance in African children treated with ACTs in order to detect the emergence of artemisinin resistance. In this study we examined the prevalence of asexual *parasitemia* following treatment with 2 leading ACTs, artemether-lumefantrine (AL) and didhydroartemisinin-piperaquine (DP), in a cohort of Ugandan children 4-5 years of age enrolled in a longitudinal clinical trial in Tororo, Uganda. Associations between pre-treatment risk factors of interest and having a positive blood smear up to 3 days after directly observed therapy were made by multivariate analysis using generalized estimating equations with adjustment for repeated measures in the same patient. A total of 202 children were included in the analysis resulting in 416 episodes of malaria treated with AL and 354 episodes treated with DP. The prevalence of *parasitemia* on days 1, 2, and 3 following initiation of therapy were 67.6%, 5.6% and 0% in those treated with AL, and 52.2%, 5.7% and 0.3% in those treated with DP. Independent risk factors for having a positive blood slide on day 1 included treatment with AL vs. DP (RR=1.34, 95% CI 1.28-1.50, $p<0.001$), having a temperature > 38.0ºC vs. ≤ 37.0ºC (RR=1.19, 95% CI 1.05-1.35, $p=0.007$) and having a parasite density > 20,000/µL vs. < 4,000/ µL (RR=3.37, 95% CI 2.44-4.49, $p<0.001$). Independent risk factors for having a positive blood slide on day 2 included elevated temperature, high parasite density, and being HIV infected. Among children living in Tororo, Uganda early parasite clearance following treatment with AL or DP was excellent with only 1 of 752 patients tested having a positive blood slide 3 days after initiation of therapy. The type of ACT given, baseline temperature, baseline parasite density and HIV status were associated with difference in parasite clearance 1 or 2 days following therapy.

170

IN VIVO EFFICACY AND SAFETY OF ARTEMETHER/LUMEFANTRINE VS. DIHYDROARTEMISININ-PIPERAQUINE FOR TREATMENT OF UNCOMPLICATED MALARIA AND ASSESSMENT OF PARASITE GENETIC FACTORS ASSOCIATED WITH PARASITE CLEARANCE OR TREATMENT FAILURE

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Drug efficacy testing has been recommended by the World health Organisation to monitor the efficacy of artemisinin based combination therapy (ACT) and possibly detect evolution/emergency of tolerance/resistance to these drugs. Currently, Artemether/Lumefantrine (ALu) is the only ACT which is being used in Tanzania and thus, testing of new ACTs such as dihydroartemisinin-piperaquine (DHA-PQ) is important because alternative drugs are urgently required. This study will be an open-label randomized trial and aims to assess the efficacy of ALu versus DHA-PQ; and the role of parasite genetic/genomic factors that might be associated with treatment outcome among patients with uncomplicated malaria treated with these ACTs. The study will be conducted from April 2013 and will recruit 600 children aged 6 months to 10 years, with uncomplicated *falciparum* malaria at Muheza Designated District Hospital and Ujiji Health Centre in Tanga and Kigoma regions, respectively (150 patients per treatment arms at each site). Follow up will be done for 68 days and the primary end point will be parasitological cure on day 28 for ALu and 42 for DHA-PQ (non-adjusted and adjusted by PCR to correct for new infections). The secondary end points will include: parasite clearance after 48 hours, parasitological cure on day 14, extended parasitological cure on day 42 for ALu and 68 for DHA-PQ, improvement in haemoglobin level at day 28 from the day 0 baseline, reduction in gametocyte carriage at day 14 and day 28 from the day 0 baseline, occurrence and severity of adverse events and genomic profile of *Plasmodium falciparum* malaria parasite. Preliminary results will be presented and discussed, and will provide important data to the National Malaria Control Program (NMCP) to be used in the ongoing review of treatment guidelines. The information will also support NMCP to recommend DHA-PQ as the second line antimalarial drug for the treatment of uncomplicated malaria in Tanzania.

171

NEUREGULIN-1/ARTEMETHER COMBINATION THERAPY PROTECTS AGAINST MURINE EXPERIMENTAL CEREBRAL MALARIA

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Cerebral Malaria (CM) is a diffuse encephalopathy caused by infection with *Plasmodium falciparum*. Despite availability of anti-malarial drugs, CM-associated mortality remains high at 30% and 25% of survivors experience cognitive disabilities and other neurological sequelae. Thus, adjunctive therapy is greatly needed to prevent CM-associated brain damage and mortality. Neuregulin-1 (NRG-1), a neurotrophic growth factor, has been shown to protect against brain injury associated with acute ischemic stroke (AIS) and acute neurotoxin exposure. The pathology of AIS-associated brain injuries shares remarkable similarities with brain injuries associated with CM pathogenesis. We hypothesized that NRG-1 will protect mice from experimental cerebral malaria (ECM)-associated brain damage while improving survival. Furthermore, we assessed the effect of NRG-1 in combination with antimalarial on survival of mice with late-stage ECM as well as specific immune determinants of ECM. To determine whether NRG-1 improves survival of mice with late-stage ECM, mice infected with *P. berghei* ANKA (PbA) were treated with recombinant human NRG-1 (1.25ng/kg) or artemether (25mg/kg) or combination of NRG-1 and artemether after onset of ECM (days 6 through 9 post-infection). We assessed effect of treatments on survival, *parasitemia*, and immune biomarkers of disease severity in PBA-infected mice. NRG-1 monotherapy reduced ECM-associated mortality by 73% compared to saline-treated mice ($p<0.01$). In addition, NRG-1 reduced systemic and brain inflammation via decrease in pro-inflammatory markers (IL-6 and IL-1α) and decrease in leukocyte accumulation in the brain respectively. However, when NRG-1 is used in combination with artemether, there is 91% improved survival in mice with ECM compared with saline-treated mice ($p<0.001$). Nevertheless, artemether alone improved survival in ECM mice by 82% ($p<0.001$). The results suggest that NRG-1 may be functioning as a CNS anti-inflammatory agent that protects against ECM pathogenesis and associated mortality and may represent a novel adjunct therapy for CM.

172

MALARIA DISCIPLINE AND NEUROPSYCHIATRIC SEQUELAE SUICIDE MORTALITY AMONG U.S. TROOPS IN VIETNAM, 1960-1975

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Malaria has influenced the outcome of numerous military campaigns from ancient times through the 20th century. Improved understanding of disease aetiology, transmission, and treatment helped to reduce the impact of the disease on effective troop strength. Field Marshall William Slim addressed this problem with a draconian model of 'malaria discipline during the Burma Campaigns of 1942-1944. The primary objective of this strategy was to reinforce combat effectiveness through malaria prevention. Failing strategies of mosquito-vector control (MVC) and personal protective measures (PPM), medical officers utilised aggressive chemotherapeutic prophylaxis and treatment methods to expedite the return of troops to full duty. American and ANZAC medical officers applied Slim's principles of 'malaria discipline' to troops who served in Vietnam between 1960 and 1975. Although the primary objective of routine chemoprophylaxis, with its attendant risk of neuropsychiatric issues, was the prevention of malaria and the bolstering of troop strength, such a routine may have exacerbated an array of endogenous and exogenous factors (e.g., pre-existing psychiatric disorders, biogenetic factors, environmental stressors, etc.) upon unanticipated increases in neuropsychiatric issues among troops who served in Southeast Asia.

173

AVAILABILITY AND DISPENSING PRACTICE OF ARTEMETHER-LUMEFANTRINE IN LOWER LEVEL PUBLIC HEALTH FACILITIES IN UGANDA

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Malaria control efforts including scaling up the availability of Artemether-Lumefantrine (AL) are on the increase. Despite all these efforts, evidence of availability and utilization of AL is quite limited. This study sought to establish whether there is any relationship between AL availability and how it is dispensed to patients with uncomplicated malaria, in selected health center IVs in Uganda. Study data extraction was carried out over

a period of 22 months (January 2011- October 2012) at the out-patient clinics of six health center IVs located in high (Aduku and Nagongera), medium (Walukuba and Kasambya) and low (Kihihi and Kamwezi) malaria transmission settings. Monthly stock levels for AL were determined by direct review of monthly drug stock cards for the study period. To assess whether there is any relationship between AL availability and how it is dispensed to patients with uncomplicated malaria, we extracted and reviewed patient charts and compared AL prescriptions with AL doses available at the health center. AL availability ranged from 171,800 doses in Walukuba to 501,39 doses in Aduku. Tis resulted in a monthly average ranging from 7809 doses to 2279 doses respectively. The first 12 months of the study period registered lower doses of AL, with three sites (Aduku, Nagongera and Kamwezi) having complete stock outs between January and April 2011. Trends showed impressive increases in AL doses in 2012, with average stocks going above 59,000 doses at each of the sites and few stockouts observed only in one site (Kamwezi) between June and July 2012. Out of the 43,420 uncomplicated malaria cases seen in the study period, 39,415 (90.8%) were prescribed AL of which 37,252 (95%) received the drug. Availability of AL has gradually increased at the selected health center, reaching optimal levels with minimal stock outs. Additionally, over 90% of patients with uncomplicated malaria were prescribed and received AL as per national guidelines.

174

FULANI SHOW DECREASED SUSCEPTIBILITY TO *PLASMODIUM FALCIPARUM* INFECTION VS MOSSI: DATA FROM A COMMUNITY-WIDE SCREENING AND TREATMENT OF ASYMPTOMATIC CARRIERS IN BURKINA FASO

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Recent data indicated that the susceptibility to *Plasmodium falciparum* infection differs for the two major ethic groups, Fulani and Mossi. Data from a recent cluster-randomized trial of community-wide screening and treatment of asymptomatic carriers of *P. falciparum* in 18 villages in Saponé, Burkina Faso, showed that the Fulani groups had a lower proportion of asymptomatic carriers (intervention arm: Fulani vs Mossi = 15.7% vs 24.4%; control arm: Fulani vs Mossi = 50.0% vs 56.9%), a lower density of asexual *P. falciparum* forms (intervention arm, mean/µl [SD]: Fulani vs Mossi = 1,780.2 [7,355.37] vs 2,107.4 [6,295.19]; control arm, mean/µl [SD]: Fulani vs Mossi = 1,137.1 [1,617.18] vs 2,321.5 [8,900.79]) and gametocytes (intervention arm, mean/µl [SD]: Fulani vs Mossi = 36.1 [81.65] vs 49.1 [410.66]; control arm, mean/µl [SD]: Fulani vs Mossi = 29.2 [30.01] vs 25.9 [69.36]) at baseline. In children under 5 years of age, lower rates of symptomatic malaria episodes with a parasite density >5,000/µl per person-year, were noted in Fulani groups compared to the Mossi groups (intervention arm: Fulani vs Mossi = 0.95 vs 1.10; control arm: Fulani vs Mossi = 0.76 vs 1.03). These data confirm previously reported differences in *P. falciparum* susceptibility between Fulani and Mossi.

175

SAFETY AND EFFICACY OF PRIMAQUINE AGAINST RELAPSE IN INDONESIAN SOLDIERS WHEN COMBINED WITH EUARTESIM, PYRAMAX OR FOLLOWING ARTESUNATE TREATMENT OF ACUTE *VIVAX* MALARIA: AN INTERIM ANALYSIS

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The safety and efficacy of primaquine against relapse by *Plasmodium vivax* hinges upon the partner blood schizontocide administered for the acute clinical attack. Following demonstrations of the safety of co-administration of primaquine with an artemisinin, piperaquine, or pyronaridine by other investigators, we commenced a randomized, open-label clinical trial of primaquine efficacy against relapse when co-administered with Eurartesim (dihydroartemisinin-piperaquine, Sigma Tau, Italy) or Pyramax (artesunate-pyronaridine, Shin Poong, Republic of Korea), or 2-days following completion of 7 days of daily artesunate therapy against asexual blood stage infection. Subjects were G6PD-normal and otherwise healthy Indonesian Army soldiers returning from 6 months of duties in highly malarious Papua to their malaria transmission-free base at Sragen, Central Java and diagnosed with infection by *P. vivax*. In addition to standard therapy applying Eurartesim, Pyramax, or artesunate, all subjects received directly observed 0.5mg/kg primaquine base as a single daily doses for 14 days and will be followed up for 12 months. At submission of this abstract, 100 subjects had been enrolled, with enrollments scheduled to continue to 180 subjects, or through July 2013, whichever comes first. Recurrent *parasitemia*s in subjects represent evidence of relapse by virtue of exclusion of re-infection and recrudescence as confounding factors, and therefore provide relatively unambiguous estimates of primaquine efficacy against relapse. We will present an interim analysis of all enrolled subjects having completed 4- to 8-months follow-up. In a previous similar study, all first relapses occurred during the first 6 months following therapy.

176

SEVERE CEREBRAL MALARIA IN NON-IMMUNE TRAVELERS RETURNING TO SLOVAKIA FROM TROPICS

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Severe malaria represents less than 10% of all malaria cases, however it is associated with high mortality of 10 - 30%, which is highest in small children and non-immune patients, e.g. travellers to hyperendemic areas. The aim of this case series was to reported severe malaria in travellers within last 10 years in Slovakia. All cases of severe malaria in travellers reported within last 10 years from inpatient department in Slovakia to Slovak Tropical Institute (STI) are reported. Only those travelling as tourist to Sub-Saharan Africa were included. During the last 10 years, eight (n=8) cases of cerebral malaria were reported. Seven of all 8 cases had deep coma (87,5%), 4 (50%) required ventilator support, 4 (50%) required

dialysis, 5 (62,5%) had liver failure and 6 (75%) had severe acidosis. All eight patients, but one, survived without sequelae and without significant toxicity, within 8 - 22 days of therapy. The one patient who died (12,5%) was treated with quinine alone, the rest of the patients were treated with artemeter, artesunate (i.m. or i.v.), with artemeter/lumefantrin or quinine with clindamycin. Severe malarial cases was rare imported diseases in Slovakia within last 10 years. In survivors were mostly leaves sequelae, e.g. deafness, epilepsy, blindness, paresis, psychomotoric sequelae. One patient treated with quinine alone died.

177

REPEATED TREATMENT WITH FIXED-DOSE ARTESUNATE-AMODIAQUINE VS. ARTEMETHER-LUMEFANTRINE IN UGANDAN CHILDREN UNDER FIVE YEARS OF AGE WITH UNCOMPLICATED MALARIA

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The safety and efficacy of the two most widely used fixed-dose artemisinin-based combination therapies, artesunate-amodiaquine (ASAQ) and artemether-lumefantrine (AL) are well established for single episodes of uncomplicated *Plasmodium falciparum* malaria, but the effects of repeated, long-term use are not well documented. This was a 2-year randomized, open-label, longitudinal, phase IV clinical trial comparing the efficacy and safety of fixed-dose ASAQ Winthrop® and AL for the treatment of uncomplicated malaria in children <5 years of age. Children in the catchment area of Nagongera Health Centre IV, Uganda with malaria due to *P. falciparum* were randomized 1:1 to receive oral ASAQ or AL in dose regimen following guidelines. Subsequent episodes of uncomplicated malaria were treated with the same medication. A total of 416 children were enrolled and experienced a total of 6033 malaria episodes (mean ± standard deviation, 15 ± 5; range, 1 - 26). For the first episodes of malaria, the PCR-corrected-cure rate for ASAQ (97.5%) was non-inferior to that for AL (97.0%). For subsequent episodes of malaria in which >100 children were enrolled (episodes 2-18), the PCR-corrected cure rates ranged between 88.1% and 98.9% per episode, with no clear difference between treatments. For all episodes, parasite clearance was 100% by day 3, and gametocyte carriage was nearly eliminated (<1%) by day 21. Treatment compliance was close to 100%. Adverse events were mainly reported during the first malaria episode, were most often related to the malaria infection or concomitant infection or injuries, and were similar between treatment groups. Anemia or neutropenia was observed in ≤0.5% of the children per episode and abnormal liver function test in 0.3% to 1.4%. All biological abnormalities resolved spontaneously with no recurrence despite high rates of treatment re-administration. In this study, ASAQ and AL were found to be similarly safe and effective for repeated, long-term use.

178

TREATING FEBRILE CHILDREN IN SUB-SAHARAN AFRICA: EVIDENCE FROM NATIONAL HOUSEHOLD SURVEYS IN MADAGASCAR, NIGERIA AND UGANDA

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Appropriate case management is a key control target for all childhood illnesses. The focus of indicators collected from population-based surveys is often restricted by illness, with little examination of polypharmacy or the use by caregivers of multiple sources of advice and treatment. Understanding how caregivers respond to an illness episode in its entirety merits further investigation. To this end, nationally-representative household surveys focused on treatment-seeking behaviour for fever among children under five were conducted in 2012 in Madagascar, Nigeria and Uganda as part of the ACTwatch program. Detailed information on treatment-seeking behaviour was collected, including where advice and treatment was sought, and diagnostic services and medicines received at each source. Unlike standard population-based surveys the ACTwatch questionnaire uses an audit mechanism that enables brand and active ingredient details to be recorded from available medicine packages, reducing the likelihood of recall bias. Treatment indicators were tabulated by country, and cross-tabulated by urban and rural location. In all three countries caregivers most commonly first sought treatment for a child's fever at home (Madagascar 44%, Nigeria 48%, Uganda 61%). Treatment was first sought from the informal private sector for 20% of fevers in Madagascar. Additional source indicators will present the number of external sources visited and the source mix. Results will be presented on the proportion of children receiving single and multiple medicines and the distribution of medicine types received from each source. For example, in Uganda 16% of children received both an antimalarial and antibiotic during their fever episode. These findings highlight how treatment-seeking for childhood illness is a dynamic process, frequently involving multiple treatments that are often sourced from several providers. This complex picture is masked by the standard approach to reporting population-based indicators. The information presented in this work has the potential to inform programming and health promotion campaigns.

179

IMPACT OF ACT SCALE-UP ON FEVER CASE MANAGEMENT IN THE PUBLIC AND PRIVATE SECTORS IN AFRICA: EVIDENCE FROM NATIONWIDE SURVEYS

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In recent years initiatives such as the pilot phase of the Affordable Medicines Facility - malaria (AMFm) have proved to be a "game changer" for the private sector, resulting in increased availability and reduced price of quality-assured ACT in for-profit outlets. Given the well-documented role of the private sector as a treatment source in sub-Saharan Africa, we investigate whether increased availability of affordable ACT is associated with improvements in case management of suspected malaria. As part of the ACTwatch program nationally-representative household surveys focused on treatment-seeking behaviour for fever among children under five were conducted in Benin, Madagascar, Nigeria, Uganda and Zambia between 2009 and 2010, and repeated in 2011/2012. Detailed information on treatment-seeking behaviour was collected, including where advice and treatment was sought, and diagnostic services and medicines received at each source. Treatment indicators were tabulated by sector across countries; differences in case management provided by each sector over time were examined using logistic regression.Increases in presumptive ACT use among children with fever were seen in all countries, ranging from 5 percentage points in Madagascar (3% to 8%) to 24 percentage points in Uganda (20% to 44%). When restricted to children who received any antimalarial the increases in ACT use were even greater, from 7% to 45% in Madagascar and 40% to 83% in Uganda, for example. These results will be further disaggregated by source of treatment to facilitate comparison with outlet survey data. During the same period in Uganda, overall availability of ACT increased from 34% to 75%, with the majority of this increase coming in the private sector. Results on appropriate case-management for fever will be contextualised in light of findings from contemporaneous outlet surveys also conducted by ACTwatch. Differences between the public and private sectors will be discussed.

180

CYP 2D6 METABOLISM IS ESSENTIAL FOR THE ANTIMALARIAL ACTIVITY OF PRIMAQUINE

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The efficacy of the 8-aminoquinoline (8AQ) drug primaquine (PQ) has been historically linked to CYP mediated metabolism. Although to date, no clear evidence exists in the literature which unambiguously assigns the metabolic pathway or specific metabolites necessary for activity, recent literature suggests a role for CYP 2D6 in the generation of redox active metabolites. In the present study, we used the specific CYP 2D6 inhibitor paroxetine to assess the effects on the production of specific phenolic metabolites thought to be involved in PQ efficacy. Further, we assessed PQ efficacy against *P. berghei* in CYP 2D knockout mice in comparison with a normal C57 background and with humanized CYP 2D6 knock in mice to determine the direct effects of CYP 2D6 metabolism on PQ activity. PQ exhibited no activity at its ED_{100} or at a dose of two times the ED_{100} in CYP 2D knockout mice, compared to 5/5 cures in normal mice at the ED_{100}. The antimalarial activity of primaquine was restored in CYP 2D knockout/ humanized CYP 2D6 knock in mice. These results unambiguously demonstrate that metabolism of PQ by CYP 2D6 is essential for antimalarial efficacy.

181

EFFICACY AND SAFETY OF ARTEMISININ COMBINATION THERAPIES FOR TREATMENT OF UNCOMPLICATED MALARIA IN HIV-INFECTED UGANDAN CHILDREN

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Artemisinin combination therapies (ACTs) are highly efficacious and safe but data from HIV-infected children receiving antiretroviral therapy (ART) are limited. We evaluated uncomplicated malaria treatment outcomes in two cohorts of HIV-infected children in Tororo, Uganda, a high malaria transmission intensity area. In the PROMOTE cohort, children were randomized to a lopinavir/ritonavir (LPV) or non-nucleoside reverse transcriptase (NNRTI; nevirapine (NVP) or efavirenz (EFV)) based ART regimen; 572 treatments for malaria with artemether-lumefantrine (AL) were given among 123 children. In the TCC cohort, 91% of children received ART (all NVP-based); 43 children were randomized to AL or dihydroartemisinin- piperaquine (DP) resulting in 201 and 165 treatments, respectively. Treatment responses and adverse events were assessed over a 28-day period following antimalarial therapy. There was over 99% clearance of *parasitemia* by day 3 and < 5% risk of recrudescence. However recurrent *parasitemia* due to new infections were common. In PROMOTE, risk of recurrent *parasitemia* following treatment with AL was significantly lower among children taking LPV-based ART compared to children taking NVP-based ART (15.3% vs. 35.5%, p=0.009). Within the NNRTI arms, recurrent *parasitemia* was 52.5% vs. 35.5%, p=0.06 in the EFV vs NVP arms, respectively. In TCC, the risk of recurrent *parasitemia* was significantly lower among children treated with DP compared to AL (8.6% vs. 36.2%, p<0.001). There were no grade 3 or 4 adverse events in TCC and 55/492 (11.2%) and 2/468(0.4%) episodes of grade 3 or 4 neutropenia and *Thrombocytopenia* respectively in the PROMOTE cohort. Antimalarial therapy with AL and DP was efficacious and well tolerated in HIV-infected children receiving NNRTI or LPV-based ART. In a setting of malaria treatment with AL, LPV-based ART regimen was associated with a lower risk of recurrent *parasitemia* compared to NNRTI-based regimens. In a setting of NVP-based ART use, treatment of malaria with DP was associated with a lower risk of recurrent *parasitemia* compared to AL.

182

CYP450 PHENOTYPING AND METABOLITE IDENTIFICATION OF QUININE BY ACCURATE MASS UPLC-MS ANALYSIS: A POSSIBLE METABOLIC LINK TO BLACKWATER FEVER

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The naturally occurring alkaloid drug quinine is commonly used for the treatment of severe malaria. Despite centuries of use, its metabolism is still not fully understood, and may play a role in the hemolytic disorders associated with the drug. Incubations of quinine with CYPs 1A2, 2C9, 2C19, 2D6, and 3A4 were conducted, and the metabolites were characterized by accurate mass UPLC-MS^E analysis. The metabolites 3-hydroxyquinine, 2'-oxoquininone, and O-desmethylquinine were observed after incubation with CYPs 3A4 (3-hydroxyquinine and 2'-oxoquininone) and 2D6 (O-desmethylquinine). In addition, multiple hydroxylations were observed both on the quinoline core and the quinuclidine ring system. Of the five primary abundance CYPs tested, 3A4, 2D6, 2C9, and 2C19 all demonstrated activity toward quinine, while 1A2 did not. Reactive oxygen species generation was also measured in human erythrocytes incubated in the presence of quinine with and without microsomes. Quinine produced robust dose dependent oxidative stress in human erythrocytes in the presence of microsomes. Taken together, these results illustrate that bio-activation of quinine resulted in the production of metabolites that increased levels of reactive oxygen species in erythrocytes. The combination of increased levels of reactive oxygen species and parasite burden likely contribute to the hemolysis associated with blackwater fever.

183

AGE-DEPENDENT CARRIAGE OF ALLELES OF *PLASMODIUM FALCIPARUM SERA5, EBA-175* AND *CSP* IN A REGION OF INTENSE MALARIA TRANSMISSION IN UGANDA

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The development of malaria vaccines is constrained by genetic polymorphisms exhibited by *Plasmodium falciparum* antigens. We investigated the age-dependent distribution of alleles or haplotypes of three *P. falciparum* malaria vaccine candidates, circumsporozoite protein (*csp*), erythrocyte binding antigen 175 (*eba*-175) and serine repeat antigen 5 (*sera5*) in a region of intense malaria transmission in Uganda. A cross sectional study was carried out between August and November 2009. Blood samples were collected after informed consent from 250 individuals below 5 years, 5-10 years and above 10 years olds. *P. falciparum* DNA was extracted from all samples. Alleles of *sera5* and *eba-175* were determined by polymerase chain reaction (PCR) amplification followed by resolution of PCR products by agarose gel electrophoresis and allele calling using photographs of ethidium bromide-stained gels. Haplotypes of CSP

were identified by sequencing 63 PCR products and using *P. falciparum* 7G8 strain sequence as a reference. Both *eba-175* FCR3 (48/178) and CAMP (16/178) alleles were observed with the FCR3 (24/67) allele being predominant among children aged below 5 years old while the CAMP (12/67) allele was predominant among older individuals. Both *sera5* alleles ORI (6/204) and ORII (103/204) were observed in the population but ORII was more prevalent. SERA*5* ORII allele was significantly associated with age (P values < 0.0001), parasite density (P value <0.0001) and clinical outcomes (P value= 0.018). There was marked CSP diversity in the Th2/Th3 region. Out of 63 sequences, 16 conformed to the reference strain and one (1/16) was similar with a West African haplotype and the majority (14/16) of the haplotypes were unique to this study region. There was an age-dependent distribution of CSP haplotypes with more haplotypes being harbored by < 5-year olds, (10/16) compared to adults (2/16). Interestingly, the CSP haplotype corresponding to 3D7 whose prototypical sequence is identical to the sequence of the leading malaria vaccine candidate RTS, S was not observed. Our data suggest that *eba-175* FCR3 allele*, sera5* ORII allele, and CSP haplotypes are targets of host immunity and under immune selection pressure in Apac District.

184

T CELL RESPONSES TO LIVER-STAGE *PLASMODIUM* ANTIGENS IN THE SETTING OF REPEATED SPOROZOITE IMMUNIZATIONS

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An effective vaccine that induces cytotoxic T lymphocytes (CTL) specific to malaria parasites could accelerate malaria eradication efforts. Sporozoite-mediated experimental immunizations induce this type of response, but the diversity of *Plasmodium* proteins has thus far prohibited the identification of sufficient T-cell antigens to develop highly effective subunit vaccines yielding sterile immunity. Over the past several years, we have developed novel high-throughput screening approaches and applied these to study the T cell repertoire of sporozoite-immunized mice. We recently identified a unique CTL response against the parasite L3 ribosomal protein. Unlike responses to the circumsporozoite protein (CSP), L3-specific CTLs are not expanded by multiple sporozoite immunizations despite normal expansion and function of these cells under other prime-boost conditions. Whereas CSP is abundant on the sporozoite, L3 is not highly expressed until the actual liver stage. The gross anti-malarial immune response induced by a single immunization with sporozoites reduces the parasite load so greatly during subsequent immunizations that L3 is never expressed and L3-specific responses can only therefore be generated during the primary exposure. Thus, although repeated sporozoite immunization expands responses to preformed antigens like CSP, this strategy may not expand CTLs targeting proteins synthesized later. Novel heterologous strategies may be needed to increase diversify CTL responses across the entire spectrum of *Plasmodium* liver stage proteins. Findings from our ongoing studies of other *Plasmodium* liver stage proteins will also be presented.

185

ANTIGEN PRESENTATION OF L3 AND OTHER LIVER-STAGE ANTIGENS IN MALARIA-IMMUNIZED MICE

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Pre-erythrocytic malaria vaccines may need to target numerous sporozoite and/or liver-stage proteins to be effective. If the protective antigens could be definitively identified, multi-component subunit vaccines could be produced. Using a novel high-throughput T cell screening system, we recently identified a CD8 response against the *Plasmodium yoelii* L3 ribosomal protein in sporozoite-immunized BALB/c mice. Unlike the $CD8^+$ T cell response against the circumsporozoite protein (CSP) that increases with each parasite exposure in our system, the L3-specific response is not boosted by repeated exposures to attenuated sporozoites. We have shown that L3-specific cells have no cell intrinsic defects that counteract their re-expansion or function but rather that broad anti-sporozoite immune responses in secondary or later exposures eliminate expression of L3, thereby preventing any opportunity for activation of memory L3-specific $CD8^+$ T cells. This T cell outcome following immunization may be emblematic of other T cells with liver-stage targets as well. Here, we studied the L3-specific T cell response in other parasite species (*P. berghei*) and other mouse strains (C57BL/6) to determine if this phenomenon was conserved in related infection models. Further, we are evaluating differences in the Class I MHC antigen presentation of L3 and CSP epitopes in the liver and are evaluating other protein targets for L3- versus CSP-like antigen characteristics following immunization. These studies may delineate malaria antigen characteristics that could predict responses to secondary immunizations, which could be useful for designing more effective malaria vaccines.

186

HBC AND HBS MODIFY DISTINCT *PLASMODIUM FALCIPARUM* BINDING INTERACTIONS

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Plasmodium falciparum is the deadliest of the human malaria parasites, and kills up to a million African children each year due to severe syndromes. Hemoglobinopathies reduce severe malaria risk, and existing data suggest that their protective effect may be related to an effect on parasite adhesion. Because HbS protects from all severe syndromes while HbC may preferentially protect against cerebral malaria, we hypothesized that host factors like HbS and HbC may differentially modify *falciparum* parasite binding to specific receptors. In assays using clinical isolates collected from children participating in longitudinal cohorts in Ouelessebogou, Mali, we identified novel endothelial molecules that support infected erythrocyte binding including extracellular matrix molecules and members of the integrin family. IE collected from children with sickle cell trait were less likely to bind to the receptor CD36 (OR 0.429 (CI 0.252-0.729), p=0.002) and Integrin $\alpha v\beta 3$ (OR 0.551 (CI 0.299-1.018), p=0.06), while IE collected from children with hemoglobin AC were less likely to bind to several other endothelial receptors E-selectin (OR 0.4 (CI 0.228-0.7), p=0.001); P-selectin (OR 0.533 (CI 0.318-0.894), p=0.02); ICAM1 (OR 0.457 (CI 0.285-0.732), p=0.01); Integrin $\alpha 5\beta 1$ (OR 0.523 (CI 0.303-0.904), p=0.02) and ICAM2 (OR 0.347 (CI 0.171-0.705), p=0.003). In summary, our results confirmed our hypothesis that different host factors differentially modify IE binding to endothelial receptors.

187

EARLY EFFECTOR CELLS SURVIVE TO GENERATE MEMORY T CELLS IN MURINE MALARIA

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Development of long-lived T cells is important for generation of memory and antigen-specific protection against repeat challenges by infectious agents including malaria. Understanding of CD4 T cell memory development is evolving, and it is especially challenging in chronic

infections such as malaria infection. We have shown that in chronic infection, central memory T cells are continuously generating effector memory, the predominant memory cell type in malaria. However, it has been challenging to phenotypically distinguish effector cells from effector memory and so, although it has been shown that CD4 effector T cells can survive into the memory phase (Harrington et al.), it was not known if $CD4^+$ effector T cells could directly generate long-lived effector memory, or if Tcm were the necessary intermediates. In the current study, we have established subsets along the spectrum of development of effector cells and use these new markers to identify effector T cells that are able to differentiate into memory CD4 T cells. Using B5 TCR transgenic malaria-specific T cells in an adoptive transfer model, as well as a T cell-specific, IFN-g reporter mouse, we demonstrate that all subsets (CD127-CD62lo or hi) have divided, and can make IFN- g, and that they are generated in murine malaria infection with IFN- g^+ TeffM dominating the peak. Furthermore, on Day 8 of infection, the early effector $CD4^+$ T cell subset (TeffE, $CD127^-CD62L^{hi}CD27^+$) has the potential to differentiate into the middle (TeffM, $CD127^-CD62L^{lo}CD27^+$) and late effector (TeffL, $CD127^-CD62L^{lo}CD27^-$) subsets, while the TeffM and TeffL are terminally differentiated (PD-1+) and express low levels of anti-apoptotic Bcl-2, and TeffL fails to maintain membrane asymmetry ($AnnexinV^{hi}$). Furthermore, our data show that of these, only the TeffE subset survives to become memory cells when transferred into antigen-naive recipients. Importantly, TeffE are able to survive and generate memory T cells (including Tcm) in naïve RAG^o recipients. Our data therefore suggest that TeffE are the elusive $CD4^+$ precursor cells that form both effector and memory subsets in response to infection.

188

ROLE OF T REGULATORY AND TH17 CELL DURING LETHAL *PLASMODIUM BERGHEI* ANKA AND NON-LETHAL *P. YOELII* INFECTION

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The outcome of malaria infection is determined, in part, by the balance of pro-inflammatory and regulatory immune responses. Host immune responses in disease including malaria may possibly finely regulated by the opposing effects of Th17 and T regulatory (Treg) cells. Male Swiss albino mice infected with *Plasmodium berghei* ANKA and *P. yoelii* respectively with 1 x 106 pRBC, in 100 ul PBS by intraperitoneal injection. Immunohistochemical analysis of Foxp3 and RORγt was observed in spleen tissue.Flow cytometric analyses of CD4+ and CD8+ T, CD4+CD25+ Foxp3+, CD4+IL-17+ RORγt+ and CD4+Il-2+ expression in splenocytes were performed on respective dpi during both the parasite infections. Western blot was performed to analyze the role of TGF-β, TNF-α, Stat-3, IL-6, Foxp3 and NFAT during the course of infection. ELISA and RT-PCR was further performed as confirmatory tests.Here we have examined the role of Treg cells and Th17 cells during malaria infection and find that low levels of Treg cells influence the outcome of infections with the lethal strain of *P. berghei* ANKA (PbA). In contrast, we observed that possibly high level of Treg cells influencing the outcome of non lethal *P. yoelii* infections. We observed decreased expressions of TGF-β, CD4IL-2 and IL-10 during PbA infection, whereas expression remains high during *P. yoelii* infection. On the other hand TNF-α, IL-6, IFN-γ and IL-23 expression is high during PbA infection and lower during *P. yoelii* infection. In combination with functional studies, we posit that Treg may convert to Th17 during PbA infection whereas; Th17 initially high during *P. yoelii* infection possibly converts to Treg cells. Thus, results from this study suggest that the critical balance between Treg and Th17 might have a key role on host pathogenesis during malaria infection.

189

IMPACT OF CHEMOPREVENTION ON THE DEVELOPMENT OF T CELL RESPONSES TO MALARIA

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Malaria-specific T cells, particularly those targeting antigens expressed during pre-erythrocytic infection, may confer protection from subsequent episodes of malaria. To test the hypothesis that selective suppression of blood-stage malaria by chemoprevention may enhance the development of T cell responses to pre-erythrocytic antigens, we performed IFNγ-ELISpot assays using PBMC samples obtained from a randomized controlled trial of chemoprevention in Tororo, Uganda, a rural area with perennial high transmission intensity. Children were randomized at 6 months of age to no therapy or monthly dihydroartemisinin-piperaquine (DP) (n=98 per group) and study drugs were continued until the infants reached 24 months of age. ELISpot assays were performed at 12 and 24 months of age using pools of overlapping peptides spanning 7 pre-erythrocytic (CSP, TRAP, LSA, SIAP1, SIAP2, CelTos, P52) and 3 erythrocytic-stage antigens (AMA1, MSP1, HGXPRT). The incidence of malaria in the no chemoprevention group was 6.95 episodes per person-year, and DP had a protective efficacy of 58% ($p<0.001$, neg binomial regression) against malaria. At 24 months of age, ELISpot responses to pre-erythrocytic antigens were detected in 23% of children on no chemoprevention and 20% of children on DP ($p=0.72$, Fisher's exact test), and responses to erythrocytic antigens were detectable in 45% and 30%, respectively ($p=0.06$). MSP1 was the most commonly recognized antigen, with responses detected in 36% of subjects at 24 months of age. Recognition of MSP1 was significantly higher among children who had been diagnosed with malaria within the preceding 30 days versus those who had been malaria-free for >90 days (57% vs. 12%, $p<0.0001$, logistic regression). However this response was not significantly associated with time to next episode of malaria after controlling for prior incidence. Although DP significantly reduces the incidence of malaria, we did not observe differences in recognition of pre-erythrocytic stage malaria antigens among young children randomized to chemoprevention.

190

ELEVATED LEVELS OF TH1 INFLAMMATORY MEDIATORS AT BIRTH ARE PROTECTIVE AGAINST PEDIATRIC SEVERE MALARIAL ANEMIA

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Severe malarial anemia remains a major cause of pediatric illness and mortality in Sub-Saharan Africa. Although the pathogenesis of SMA is not fully understood, there is evidence that perturbations in the timing and magnitude of the innate immune response may influence whether a *Plasmodium falciparum* infection triggers a protective or pathogenic outcome for the host. To understand whether specific individuals are predisposed to SMA due to a cytokine production pattern established from birth, the associations between cord blood cytokines and SMA, defined as a hemoglobin <60g/L in the presence of a malaria infection, during the first four years of life were evaluated in a birth cohort in Muheza, Tanzania. Levels of tumor necrosis factor (TNF), TNF receptors

I and II (TNF-RI and TNF-RII), interleukin (IL) 1β, IL-4, IL-5, IL-6, IL-10, and gamma-interferon (IFN-γ) were measured in cord blood samples obtained from 781 participants, including 71 who experienced SMA episodes. Cox Proportional Hazard Models with shared frailties were used to calculate floating absolute risks to assess the shapes of associations between SMA and cytokine, receptor, or cytokine ratio levels. SMA risk decreased progressively across increasing levels of cord blood TNF, TNF-RI, IL-1β, and, in contrast to the findings consistently observed during acute SMA episodes, the ratio of TNF to IL-10. The risk for SMA did not vary substantially over quartiles of TNF-RII, IL-5, IL-6, and IL-10 nor between undetectable and detectable levels of IL-4 and IFN-γ. The fully adjusted hazard ratios for SMA per 1 standard deviation change of log-transformed TNF, TNF-RI, and IL-1β were respectively: 0.83 (0.70, 0.99), 0.74 (0.64, 0.87), and 0.59 (0.49, 0.70), and these associations did not vary substantially when stratified across the participant level characteristics of sex, transmission season at delivery, insecticide-treated net use, thalassemia, sickle cell trait, birth weight, maternal gravidity, maternal age, and placental malaria at delivery. In summary, these findings suggest that infants with high cord blood levels of the Th1 inflammatory mediators TNF, TNF-RI, and IL-1 are protected against SMA in early life and that there may be a specific role for inflammatory cascades in the chronic prevention of SMA that is independent of the dysregulation in inflammatory mediators that develops acutely during an infection.

191

THE RECEPTOR TYROSINE KINASE EPHB2 IS INVOLVED IN LIVER DAMAGE DURING RODENT MALARIA INFECTION

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Eph receptors and their Ephrin ligands represent the largest family of receptor tyrosine kinases. This family of molecules is divided into EphA and EphB receptors that bind to Ephrin A and Ephrin B ligands, respectively. Beyond their well-defined role in developmental processes, cell motility, cell trafficking/adhesion and their implication in cancer, nothing is known about their activation during malaria infection. We sought to investigate whether the EphB receptors were modulated during malaria infection and explored their involvement in disease pathogenesis. Infection with both *Plasmodium berghei* ANKA and *P. chabaudi* AS led to a significant upregulation of EphB2 and EphB3 mRNA and proteins in the liver. EphB2-/- mice were protected from liver damage during infection with both species as measured by collagen deposition and levels of circulating liver enzymes, despite a similar parasite burden in the livers of *EphB2-/-* and littermate control mice. This protection was correlated with an absence of leukocyte infiltration in the liver of *EphB2-/-* mice. In addition transcription of proinflammatory cytokines, chemokines and adhesion molecules were downregulated in the livers of *EphB2-/-* compared to WT littermate control mice. To determine if this upregulation of EphB2 trasncription in the livers of infected mice could be transcripts in infiltrating haemotopoietic cells we FACS sorted splenocytes from infected mice into CD4+T cells, CD8+ T cells, CD11c+ dendritic cells and CD11b+ macrophage / monocyte / neutrophil subsets. Transcription of EphB2 was found to be upregulated on CD11b+ cells subset at day 2 post-infection. This data suggest that EphB2 may contribute to malaria parasite-induced liver damage by mediating the accumulation of CD11b+ leukocytes in the liver in rodent malaria infections.

192

THE INFLUENCE OF *IN UTERO* EXPOSURE TO *PLASMODIUM FALCIPARUM* ANTIGENS ON SUSCEPTIBILITY OF CAMEROONIAN INFANTS TO MALARIA DURING THE FIRST YEAR OF LIFE

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The developing fetus may be exposed to malaria antigens transferred through the placenta from the maternal circulation and produce an antibody response. Currently, the nature this response and its impact on immunity to malaria in infants are not well understood. We studied a cohort of 361 babies at birth and through their first year of life in a malaria holoendemic region of Cameroon. *In utero* malaria-specific antibody response was assessed by detection of immunoglobulin M (IgM) in cord blood, to a panel of eight malaria antigens, since maternal IgM does not cross the placenta. A newborn was considered to have responded *in utero* if IgM to at least two malaria antigens or malaria parasite-infected erythrocyte extract, were detected using a multiplex analyte platform (MAP) assay. Cord blood collected from United States babies was used as negative controls. Microscopy and nested PCR were used for malaria parasite detection. Overall, 10.5% of the newborn babies had IgM in their cord blood that recognized a range of blood-stage antigens, including vaccine candidates on the surface of merozoites. The IgM response was significantly higher in babies born to mothers with high placental malaria parasite density ($p=0.042$), and lower when mothers received Intermittent Preventive Treatment with Sulfadoxine-Pyrimethamine ($p<0.001$). Infants who were exposed to malaria *in utero* and produced malaria-specific antibodies before birth had significantly higher rates of *P. falciparum* infections by microscopy ($p=0.01$) and PCR ($p=0.02$) during their first year of life than those without evidence of prenatal exposure and IgM response. These data show that some prenatally exposed babies in malaria-endemic regions mount specific antibody responses to malaria antigens *in utero* but become more susceptible to malaria during the first year of life.

193

IMPACT OF CHEMOPREVENTION ON THE FREQUENCY OF FOXP3+ CD4 REGULATORY T CELLS

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Plasmodium falciparum infection has long been known to possess immunosuppressive properties. The mechanisms behind this suppression in human infection are largely unknown, but may be related to induction of immunoregulatory cell populations such as FoxP3$^+$ CD4 T_{regs}. To test the hypothesis that selective suppression of blood-stage malaria by chemoprevention limits the induction of FoxP3$^+$ T_{regs}, we quantified T_{regs} in whole blood samples obtained from children enrolled in a randomized controlled trial of chemoprevention in Tororo, Uganda, a rural area with perennial high transmission intensity. Children were randomized at 6 months of age to monthly dihydroartemisinin-piperaquine (DP) or no chemoprevention (n=98 per group). Study drugs were continued until the infants reached 2 years of age, at which point 100 µL of whole blood was obtained and stained with surface antibodies to CD3, CD4, and CD25. Following fixation and permeabilization, intracellular staining was performed for FoxP3. Samples were analyzed on an Accuri C6 cytometer in our field laboratory, and analyzed using FlowJo software. Statistical analyses were performed using Wilcoxon rank sum

and Spearman correlation. The incidence of malaria in the group not receiving chemoprevention was 6.95 episodes per person-year, and DP had a protective efficacy of 58% (p<0.001) against malaria. The median frequency of FoxP3$^+$ T_{regs} was 5.28% in the no chemoprevention group vs. 6.14% in the DP group (p=0.16). Interestingly, there was a strong inverse correlation between the prior incidence of malaria and the frequency of FoxP3$^+$ T_{regs} (r= -0.32, p=0.006). In addition, there was a positive correlation between the time since last malaria episode and the frequency of FoxP3$^+$ T_{regs} (r=0.24, p=0.04). Although DP significantly reduces the incidence of malaria, it did not significantly alter the frequency of FoxP3$^+$ T_{regs}. Our findings suggest that the frequency of circulating FoxP3$^+$ T_{regs} is higher among children who have experienced relatively few episodes of symptomatic malaria, suggesting a possible immunomodulatory role.

194

DELINEATION OF THE IMPACT AND MARKERS OF ASYMPTOMATIC MALARIA PARASITEMIA IN THE CONTEXT OF IPT IN PREGNANT WOMEN AT DELIVERY IN CAMEROON

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To evaluate the impact of asymptomatic malaria during pregancy, we assessed cytokine production and hematological parameters using peripheral, placental blood and impression smears of placenta collected from 140 women at delivery in suburban Yaounde. 79.3% of the women took IPT during pregnancy and 57.66% of those women also slept under bed nets. There were no differences in the prevalence of asymptomatic *parasitemia* and anemia between women who took IPT and those who did not (19.92% vs. 24.14%, and 16.22% vs. 27.59% respectively). However, anemia was significantly associated with asymptomatic *parasitemia* in both groups (p = 0.0001 and p = 0.0079, respectively). In addition, gestational week was significantly shorter (p = 0.03) and baby's weight was significantly smaller in parasitemic women. Placental serum levels of IL-10 and CXCL-10 were significantly higher in parasitemic women (p = 0.001 and p = 0.02, respectively), peripheral serum levels of IL-10, CXCL-10 and IL-10 /IFN-γ ratio was significantly in parasitemic women (p < 0.0001, p = 0.0069 and p = 0.04, respectively). The percentage of neutrophils and monocytes on placental impression smears and the number of monocytes per ml of peripheral and placental blood were significantly higher in parasitemic women (p = 0.0091, p < 0.0001, p = 0.02 and p = 0.0075, respectively). Low Hb (r = -0.4, p < 0.0001), high IL-10 levels (r = 0.3, p = 0.0007) and the number of monocytes (r = 0.5, p < 0.0001) in peripheral blood were significantly associated with placental malaria. Baby's weight was associated with placental malaria (r = -0.2, p = 0.024), placental levels of IFN-γ (r = 0.2, p = 0.014), CXCL-10 (r = -0.3, p = 0.0006). In addition, preterm deliveries were significantly associated with peripheral and placental levels of CXCL-10 (r = 0.24, p = 0.0059 and r = 0.31, p = 0.0002, respectively), placental levels of IL-17A (r = 0.23, p = 0.0052), the percentage of neutrophils and lymphocytes in placental blood (r = -0.29, p = 0.0005 and r = 0.29, p = 0.0004). Anemia was significantly associated with peripheral plasma levels of IL-10 (r = 0.24, p = 0.0051), CXCL-10 (r = 0.2, p = 0.04) and placental serum levels of CXCL-10 (r = 0.27, p = 0.0011). Our data suggest that despite the use of IPT during pregnancy, asymptomatic malaria is prevalent during pregnancy and peripheral serum levels of IL-10 and CXCL-10 can be used as markers of asymptomatic malaria.

195

HIGH MALARIA TRANSMISSION INTENSITY AND ADVANCING AGE ARE ASSOCIATED WITH LOWER AVIDITY TO MSP-1 IN 3 CROSS SECTIONAL STUDIES IN UGANDA

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Humoral immunity is critical in modulating morbidity and mortality from *falciparum* malaria. Unfortunately, protection from clinical disease takes many years to develop, during which time children living in endemic areas experience multiple episodes of symptomatic malaria. Avidity of anti-*falciparum* antibodies may play an important role in protection, but little is known about how avidity is associated with malaria transmission intensity and age. We evaluated antibody avidity to merozoite surface protein-119 (MSP-119) by ELISA using serum eluted from dried blood spot samples obtained from 3 cross-sectional surveys conducted in regions of Uganda with varied transmission intensity (Jinja, EIR=3; Kanungu, EIR=21; Tororo, EIR=305) in 2012. The 583 subjects with detectable IgG antibodies to MSP-119 ranged in age from2 to 87 years and came from all 3 studied regions. Antibody avidity was evaluated by ELISA including a step in which guanidine HCl was added to disrupt binding of low avidity antibodies. An avidity index was calculated by dividing reactivity with the disruption step to reactivity in a parallel assay without the disruption step. In a multivariate analysis including age and varied transmission study site, the avidity index for IgG binding to MSP-119 was significantly lower in Tororo (the highest transmission site) compared to both other sites (p<0.0001 for both comparisons). In addition, we observed that avidity index was independently associated with age, gradually increasing at all 3 sites to 55 years of age, but then declining (p=0.038 for difference in slopes). These data suggest among several possible explanations that a high intensity of malaria transmission may adversely affect affinity maturation of anti-*falciparum* antibodies. In addition, these data suggest that effective immune responses may wane in people over the age of 55, a rarely studied demographic in terms of malaria immunity. We plan to corroborate these findings by evaluating a larger number of serologic responses in representative cohort studies from the same 3 sites.

196

EXPRESSION OF MARKERS OF TISSUE DAMAGE AND INFLAMMATION AND TH1 AND TH2 CYTOKINES IN PLACENTAS WITH MALARIA INFECTION IN AN ENDEMIC AREA OF COLOMBIA, 2009-2011

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Pregnancy is a special state of immunomodulation which favors microbial infections. Globally, at least fifty million women are at risk of malaria during pregnancy. Pregnant women are the group with the second highest rate of mortality from malaria. Presence of *Plasmodium* in placental tissue is associated with alterations in the fetal-maternal interface, which have consequences on the development of the gestation and the fetus, including abortion, pre-term delivery, low birth weight and low hemoglobin in the newborn. The changes in placenta are closely related to development of hipoxia and presence of mononuclear infiltrates which regulate the cytokine profile, and, therefore, affect the immune normal status of the placenta required for a successful gestation. In addition, recent reports highlight the immune-stimulant role of hemozoin on the syncytiotrophoblast via the action of chemokines upon mononuclear

recruitment facilitation in the placenta. This study will be carried out in a malaria-endemic region of Colombia and established fundamental knowledge to enhance our understanding of the pathophysiology of placental malaria, specifically with respect to the presence of hypoxia, apoptosis and cytokine profiles in placental tissues of pregnant women with malaria. The proposed methodology is based on the analysis of samples from Puerto Libertador- Tierralta, and Montería, Córdoba. Three groups were assessed: 1) 20 pregnant women with *P. vivax* placental infection, 2) 10 pregnant women with *Plasmodium falciparum* placental infection, and 3) 30 pregnant women without placental infection. The expression of markers associated with hypoxia and inflammation was by real-time PCR of the genes: HIF-1, VEGF, COX-2 and COX-1, the measure the expression of IL-2, IL-4, IL-10, TNF-α, IFN-γ by real time PCR and the apoptosis was detected by DeadEndTM Colorimetric TUNEL System. Our results confirm the predominance of pro-inflammatory cytokines in pregnant women infected with malaria, as well as significant inflammatory increase determined by increased expression of COX-1 and COX-2 genes in placental tissue, regardless of the infecting species, we observed differences in the inflammatory status and the induction of apoptosis between infected and uninfected placentas.

197

DIFFERENTIALLY REACTIVE ANTIGENS BETWEEN *PLASMODIUM FALCIPARUM* INFECTED ASYMPTOMATIC AND SYMPTOMATIC INDIVIDUALS IN THE PERUVIAN AMAZON: A GENOMIC SCALE COMPARISON

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Asymptomatic *Plasmodium falciparum* infections are a problem in low transmission regions like Brazilian and Peruvian Amazonia because of their potential for maintaining transmission. Paradoxically, immunity in Amazonia has been observed after few malaria infections (asymptomatic *Plasmodium parasitemia* implies clinical immunity) in contrast to high transmission regions where acquired immunity takes years and intense seasonal or continuous transmission to develop. Antibody-dependent mechanisms play an important role in the reduction of *parasitemia* and can diminish clinical symptoms as demonstrated by passive transfer of hyperimmune immunoglobulin G. We tested the hypothesis that *P. falciparum* proteins are differentially recognized by Asymptomatic and Symptomatic parasitemic individuals. Health post-based passive surveillance was used to identify patients with symptomatic *P. falciparum* malaria (n=24) and index patient-based active case detection was used to find asymptomatically infected subjects (n=14). *P. falciparum* protein arrays containing 824 proteins (downsized selected for reactivity from arrays containing > 1000 proteins) were probed with subjects' sera. Signal values were transformed by variance stabilization and Bayes regularized t-test was used to compare groups. Results were corrected for False Discovery Rate. 535 antigens passed the reactivity cutoff, of which 52 were differentially reactive (p-value <= 0.01). Parasitemia differed between groups (means: Symp = 7325 p/ul, Asymp = 526 p/ul, p-value < 0.001). Overall reactivity was higher in asymptomatic individuals; nevertheless, a set of 5 proteins was highly reactive for both clinical conditions, making these candidates for sero-epidemiological surveillance. Given the parasite load and clinical conditions we expect the 52 differentially reactive antigens to be important in the development of immunity. Vaccine candidates MSP-1, EBA175 and LSA-3 were identified, and interestingly 13 of the antigens are conserved *Plasmodium sp.* proteins with unknown functions. These results support multi-protein targeting as a more efficient way of vaccination, even in a low transmission rate scenario.

198

PRIMARY EBV INFECTION IN INFANTS FROM A MALARIA HOLOENDEMIC REGION OF KENYA RESULTS IN ELEVATED EBV-LYTIC-SPECIFIC IFN-Γ T CELL RESPONSE

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Cytotoxic T lymphocyte responses are important in controlling Epstein-Barr virus (EBV) replication. Previous findings indicate that impaired EBV-specific IFN-γ CD8+ T cell responses and elevated viral load appear in individuals from malaria-holoendemic regions. However, IFN-γ T cell responses following primary EBV infection in infants from malaria-holoendemic regions are poorly understood. We evaluated EBV-specific latent and lytic IFN-γ T cell recall responses in peripheral blood mononuclear cells of HLA Class I genotyped infants from two geographically proximate regions in western Kenya; Kisumu (malaria-holoendemic) and Nandi (malaria-hypoendemic) at 12, 18 and 24 months of age using enzyme linked immunospot assay (ELISPOT) while EBV viral load were determined by real-time quantitative PCR. At 12 months of age, more Kisumu infants (59%) had IFN-γ ELISPOT responses to EBV-lytic-epitope peptides relative to Nandi infants (14%) and a two orders of magnitude higher EBV load, in addition EBV load positively correlated with EBV-lytic-specific IFN-γ ELISPOT responses. However, there were no variation EBV-latent-epitope peptides response based on either study-site or age. In conclusion, our data demonstrates that elevated EBV load during the first year of life induces EBV-lytic-specific IFN-γ ELISPOT responses that appear to quickly wane. This has implications for the establishment of immunologic memory necessary to control persistent EBV infections.

199

QUALITY OF MALARIA CASE MANAGEMENT AMONG CHILDREN UNDER FIVE YEARS AT LOWER LEVEL HEALTH FACILITIES IN TORORO DISTRICT, UGANDA

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Early diagnosis and prompt effective treatment of cases is a key malaria control strategy. Effective case management entails quality in diagnosis and treatment of cases. We assessed the quality of malaria case management among children under the age of five years at lower level health facilities in Tororo district, Uganda. The study was conducted at 3 level IV and six level III public health facilities. Health workers were assessed while managing 384 children with suspected malaria. Quality of malaria case management was assessed against national guidelines. Caretakers' attitudes about the quality of malaria case management and their understanding of instructions given by health workers were assesses at exit interviews. Key informant interviews were conducted with 8 health facility heads. A composite index was utilized to determine the optimal quality of malaria case management and its predictors. Clinicians took adequate history of the sickness in 75.3% (289/384) of the cases. Temperature and weight were measured in 57.3% (220/384) and13.0% (50/384) of the cases respectively. Parasitological diagnosis was performed in 48.4% (186/384) of the cases. Majority 82.6% (317/384) of the children were prescribed an ACT for malaria. Quality of care was optimal in 46.6 % of the cases. Optimal care was significantly associated with supervision of health workers in the last 6 months prior to the survey (AOR=4.7; 95% CI 2.0- 10.9), adequate understanding of instructions by caretakers (AOR=5.6; 95% CI 2.1 - 14.9), getting care from a level IV health facility (AOR=6.3; 95% CI 3.8 - 10.2) and treating older children (AOR= 2.5; 95% CI 1.3 - 4.8). Quality of malaria case management among children under five years was largely suboptimal. Quality of care was influenced by training and supervision of health workers, adequacy of infrastructure at the facilities and health system factors. The Ministry of Health and

district leaders should ensure continuous training and supervision of health workers and provide the required infrastructure to improve quality of malaria case management.

200

ARTEMISININ-BASED COMBINATION THERAPY: KNOWLEDGE AND PERCEPTIONS OF PATENT MEDICINE DEALERS IN OWERRI METROPOLIS, IMO STATE, NIGERIA AND IMPLICATIONS FOR COMPLIANCE WITH CURRENT MALARIA TREATMENT PROTOCOL

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This study was done to access the knowledge and perceptions of Patent Medicine Dealers in Owerri Metropolis of Nigeria about Artemisinin Based Combination Therapy as first line treatment for malaria using structured pre tested questionnaires administered to 80 randomly selected consenting respondents. About 67.5% and 32.5% of males and females respectively participated in the study. Most of them (56.3%) had secondary school education with about 50% having five to ten years experience in the business. The level l of knolwledge was shown to be high (82.5%), with 81.3% had proper understanding of the term "artemisinin-based combination therapies" and 80% knowing the correct dosage for artemisinin-based combination therapies. But despite the level of awareness, only 32.5% knew the correct timing for administration of the drugs. The result of this study showed no significant relationship between the level of knowledge and either educational attainment ($\chi 2 = 4.889$, df=4, p value=0.558) or the years of experience ($\chi 2 = 29.095$, df=4, p value=0.000) althogh knowledge improved a bit as expecience increased. 93.8% in the study reported that ACTs are more effective than other anti-malarial drugs. The quantity of ACT available on counters are low and there is no significant relationship ($\chi 2 = 18.833$, df=6, p value=0.004) between the avilabilty of ACT and the quantity of ACT available in stock at the time of this study. This study shows that awareness on artemisinin-based combination therapies has improved among Patent Medicine Dealers, even though other anti-malarial drugs are still in use and are marketed by them. It becomes necessary that efforts towards awareness be scaled up with emphasis on recommended time of administration and correct prescription to enhance and sustain intermittent presumptive treatment as an effective method of malaria control since this group of people still provide the major access to drugs in Nigeria and other topical endemic areas.

201

TEST-BASED TREATMENT OF MALARIA - POTENTIAL BARRIERS TO EFFECTIVE IMPLEMENTATION AMONG UNDER-FIVE CHILDREN IN RURAL GHANA

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World Health Organization guidelines now require that all cases of malaria be confirmed through test before treatment is started. We investigated the potential barriers to effective implementation of the policy among under-five children in rural Ghana. We used a mixed methods approach to evaluate treatment outcomes for malaria and non-malaria febrile illnesses managed using the revised approach, assessed adherence to current guidelines for the management of under-five childhood illnesses as proxy indicator of health worker adherence to the new policy and assessed the acceptability of the revised guideline to caregivers. Treatment outcomes for malaria and non-malaria febrile illnesses differ significantly in terms of recovery from fever, anemia and in caregiver perception of treatment outcomes, with poorer outcomes for children with non-malaria fevers. Health worker adherence to current guidelines is poor. Respiratory rate is checked in only 4% of children. Out of the 11 required tasks, it is in only 35% of children that more than 6 tasks are performed. All 11 tasks were performed in only 1% of children. Caregiver acceptance of test-based treatment of malaria is however high (98% of caregivers). Factors that promote caregiver acceptability include the perception that blood test represents improvement in the quality of care and is likely to lead to improved treatment outcomes. Implementation of the revised guidelines in rural Ghana is likely to be bolstered by high caregiver acceptability, but undermined by poor health worker adherence to guidelines. Any perception that it leads to poorer treatment outcomes for children with non-malaria fevers could undermine acceptability. Improvement in the management of non-malaria fevers is important for effective implementation of the new policy.

202

A COMPARISON OF KNOWLEDGE, ATTITUDES, PRACTICES AND BEHAVIORS ON MALARIA IN AREAS RECEIVING 'INTENSE' VS. 'NON-INTENSE' BCC INTERVENTIONS IN AN ARTEMISININ RESISTANCE SETTING, WESTERN CAMBODIA

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In Cambodia, Behaviour Change Communication (BCC) campaigns represent an integral component of previous and ongoing malaria efforts aiming at fighting artemisinin resistant parasite and moving towards malaria elimination. These include broadcasting malaria prevention, treatment and diagnosis messages via TV, radio and mobile broadcasting units (MBUs), the distribution of Information Education and Communication (IEC) materials, and the introduction of Mobile Malaria Workers (MMWs) in malaria at risk villages. In order to look at the potential added effect of 'intense' BCC interventions in three Western provinces an assessment was conducted in Dec 2012 two years after start of BCC implementation. 'Non-intense' BCC (niBBC) interventions (e.g. radio or TV) were compared to "Intense" BCC (iBBC) through VMWs, VHVs, mobile broadcasting units, and listener viewer clubs. The hypothesis was that villages (household respondents) receiving iBCC interventions were more likely to improve their knowledge, attitude and practice with regard to malaria compared to those villages (household respondents) only receiving niBCC messages. A stratified multi-stage cluster sampling approach was used; 30 villages were visited (15 in each stratum) and a total of 774 households were interviewed. The comparison between intense versus non intense BCC intervention revealed several positive outcomes: (i) iBCC intervention resulted in a decrease of about 10% of wrong beliefs related to malaria transmission mode; (ii) iBCC rose in a 18% increase in promptness of treatment (within 24 hours of onset of fever) among households with any fever case; (iii) iBCC resulted in a 15% increase in discussion about malaria within the community, 13% increase in awareness of appropriate source for health care and a 9% increase of awareness of the danger of monotherapies; and (iv) iBCC resulted in a 5% increase of knowledge of key messages. This study shows evidence of improved levels in behaviour enpoints and not just on knowledge endpoints as usually reported in BCC studies. In addition, recommendations and lessons learned from this assessment might be very valuable to the national malaria programme with regards to the planning and implementation of future effective BCC interventions since a number of behavioural factors are thought to contribute to the emergence and spread of drug resistance in this region.

203

ECONOMIC BURDEN OF MALARIA ON FARM INCOME AMONG RURAL HOUSEHOLDS IN BENUE STATE, NIGERIA

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The economic burden of malaria on rural farm income was determined. Determinants of farm income - labor loss, cost of malaria control, cost of malaria prevention, cost of malaria treatment, and cost of transportation to treatment centers - significantly affected the rural farm income. Rural households should be empowered to participate in the National Health Insurance Scheme of Federal Government of Nigeria.

204

IMPROVING MALARIA LABORATORY DIAGNOSIS AT UGANDA MALARIA SURVEILLANCE PROJECT (UMSP) SENTINEL SITES

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Parasitological confirmation of malaria diagnosis is currently recommendation world wide in all patients suspected of having malaria before treatment with Artmisinin combination therapy. This emphasizes the importance of laboratory diagnosis of malaria by microscopy in current case management of disease. Though, we still have gaps in our health facilities which need to be improved to produce defendable and believable results. This study was undertaken to improve accuracy of malaria diagnosis by microscopy by giving refresher training course, supplying quality reagents, onsite mentoring and re-reading the stained slides by expert laboratory technician in UMSP sentinel sites in Uganda. Sentinel sites laboratory needs assessment was conducted in 12 health facilities. Gaps were indentified and addressed accordingly. UMSP provided quality reagents, SOPs, bench aids, improved on number of lab personnel, onsite mentoring and sensitization of clinician on treating patients with confirmed laboratory diagnosis. Site laboratories were switched from Field's to Giemsa stain. Laboratory personnel were re-trained on staining thick and thin smears, mounting, labeling, organizing and storage of slides examined. Slide quality and accuracy of results were assesses on individual basis. All sites now refer over 95% of suspected malaria cases for confirmatory tests in the laboratory because clinician believe quality results are produced from the health facilities labs. For slide re-checking there is great improvement in accuracy though there is still some significant variation on the three parameters: sensitivity, specificity and percentage agreement across sites. In conclusion, insuring presence of supplies, refresher training, on site mentoring and slide re-checking enhanced the accuracy of malaria diagnosis by microscopy in all UMSP sentinel sites.

205

MODELING BEHAVIORAL, ENVIRONMENTAL AND EPIDEMIOLOGICAL FACTORS THAT INFLUENCE THE UPTAKE OF "TEST AND TREAT" POLICIES FOR MALARIA IN A RURAL DISTRICT OF SOUTHWESTERN SENEGAL

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Recently, global malaria treatment policy has changed from presumptive treatment based on symptomatology to targeted "Test and Treat" (T&T) with rapid diagnostic tests (RDTs) and artemisinin combination therapy (ACT). This transition involves changing long-standing behavior among health providers and patients, which results in a delay between the introduction and full implementation. Our objective is to understand the behavioral processes underlying the transition to suggest approaches for accelerating uptake of T&T. In order to evaluate how local treatment practices respond to national malaria policies, we examine detailed records for the period 2000-2011 from health clinics in Oussouye, Senegal (total fever cases, n=115,432)., where malaria is mesoendemic We show that although there may be long lead-times after national policies are created, once health providers begin to implement T&T in a fraction of cases, full adoption happens rapidly. The behavioral response for T&T uptake is well-described by the logistic function, suggesting that growth in confidence in testing results begins slowly before transitioning to a phase of rapid growth and, finally, saturation. Although the transition can occur rapidly, the initial delay interferes with the adoption of T&T policies. Health providers' belief in accuracy of RDTs precedes full adoption of T&T, suggesting this as a behavior to target to expedite T&T uptake. When malaria tests are unavailable, or not administered, health providers compensate by relying on environmental predictors (i.e. rainfall) when choosing to administer antimalarials but these treatments do not correlate closely with observed incidence. This overdiagnosis emphasizes the importance of speeding up transition to T&T. Our results suggest that national policies alone are insufficient to guarantee the adoption of T&T policies, and policy makers should encourage education that focuses on improving health providers confidence in test results. Accelerating T&T uptake in new regions will reduce overdiagnosis and costs in mesoendemic areas.

206

HARMONIZING MALARIA IN PREGNANCY GUIDANCE: THE PATH OF LEAST CONFUSION

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Thirty-nine countries in sub-Saharan Africa have malaria in pregnancy (MIP) policies in place, including intermittent preventive treatment (IPTp), insecticide treated bed-nets (ITNs) and effective case management. Nonetheless, IPTp and ITN coverage among pregnant women remains well below international goals. MIP policies are typically produced by National Malaria Control Programs (NMCP), but are implemented by National Reproductive Health Programs (RHP). We reviewed MIP policy documents from the NMCP and RHP in Kenya, Mali, Mozambique, Tanzania and Uganda to understand 1) how closely national MIP documents reflect 2007 WHO MIP guidance and 2) how consistent documents produced by the NMCP and RHP are with each other. We developed a framework to compare MIP documents from RHP and NMCP according to WHO guidance for MIP, including IPTp timing and dosing, directly observed therapy, linkages to HIV prevention programs, promotion and distribution of ITNs, and diagnosis and treatment. All countries have national

documents promoting IPTp, ITN use, and case management of MIP. WHO guidance was not always reflected in these documents: four countries restrict dosing of the first and second IPTp doses to specific gestational weeks, provide inconsistent guidance on MIP prevention in HIV+ women, and fail to provide clear guidance on the different antimalarial treatment that should be administered in the first vs. later pregnancy trimesters. All countries had discordant guidance between RH and NMCP in at least one official MIP guidance document. For example, all countries had conflicting guidance on the timing or dosing of SP and the mechanism pregnant women should use to obtain ITNs. In conclusion, considerable discrepancies exist between MIP guidance documents from NMCP and RHP. These discrepancies contribute to confusion by health workers implementing MIP programs, contributing to the low coverage of IPTp and ITNs. Harmonization of national MIP documents is urgently needed, with effective re-orientation and supervision of health workers to updated materials to help accelerate implementation. This exercise should be repeated in other malarious countries.

207

SCHOOLS, COMMUNITIES AND HEALTH FACILITIES: ENSURING CONTINUED ACCESS TO LLINS

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Continuous distribution of long-lasting insecticidal nets (LLINs) is necessary to sustain universal coverage of LLINs. As soon as a mass distribution campaign ends, net ownership begins to decline as nets wear out and the population grows. Unless net ownership is maintained through carefully planned continuous distribution systems, losses in coverage will eventually be followed by increased malaria morbidity and mortality. Therefore, mechanisms that provide a continuous supply of replacement LLINs should be integrated into all national LLIN strategies, including: integration of net distribution with ANC and EPI clinic visit services, short-term seasonal school-based distributions, and consumer "pull" mechanisms, by which informed and empowered consumers obtain replacement nets from commercial vendors with potential for private sector subsidies. NetWorks has assisted 15 countries in developing continuous distribution strategies. Five countries have begun piloting one more channels, including Tanzania, Ghana, Nigeria, South Sudan, Senegal and Madagascar. Through a participatory process with national stakeholders, NetWorks uses a mathematical modeling tool, NetCALC, as well as operational feasibility assessments, to design continuous distribution strategies at national and subnational level. Interim results from pilots show that distribution through schools could be a valuable additional channel for maintaining universal coverage over the coming years in areas where the gross attendance ratio for primary school is over 80%. Where school and health facility access is limited, community distribution provides an alternative mechanism for delivering nets to households that need them. Results from household surveys and lessons learned from South Sudan, Nasarawa State Nigeria, and Madagascar will be presented. In Ghana and Senegal, multiple channels are being combined to reach a wide array of target groups; the operational and financial impact of implementing multiple distribution channels will be discussed.

208

PECADOM PLUS: INCREASING CARE ACCESS AND DECREASING MORBIDITY IN RURAL SOUTHEAST SENEGAL THROUGH ACTIVE, HOME-BASED SURVEILLANCE AND TREATMENT OF MALARIA

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Malaria is endemic throughout Senegal, and in rural areas, physical, financial, and educational barriers limit care access. Though Senegal's home-based malaria management program (PECADOM) effectively addresses many of these challenges, numerous cases remain untreated because care is not sought. During the 2012 rainy season, Peace Corps, PMI and the Senegalese health system piloted an active case detection model called PECADOM Plus in five villages in southeastern Senegal. During the pilot, one home-based care provider (HCP) from each of the target villages was trained to conduct weekly, village-wide house-to-house sweeps to identify all fever cases, diagnose using malaria rapid diagnostic tests, and treat uncomplicated cases with artemisinin-based combination therapy. Village-elected care groups were trained to assist the HCPs in recognition of fever cases during active sweeps. Though resource constraints limited weekly sweeps to one village, a baseline sweep was conducted in all five villages and an end of season sweep in three of the five villages. Data were compiled from records of weekly sweeps and health post registers from 2008-2012. In total, 563 people were tested and the 404 positive, uncomplicated cases received free treatment for malaria. During the baseline sweep of all villages in July, HCPs treated 87 malaria cases compared to 54 at the health post during the entire month. Then after four months of weekly sweeps in one village, malaria prevalence was 88% lower than in two comparison villages during the end of season sweep. While the number of uncomplicated malaria cases increased at the health post, the proportion of severe cases decreased 41% from the previous years. Weekly active detection and management of fevers by HCPs increased access to care and reduced malaria severity. Additionally, the community-based care groups encouraged early treatment seeking, reducing severe malaria at the health post. PECADOM Plus should be tested on a larger scale and could be an important tool in Senegal's repertoire of malaria interventions.

209

CROSS SECTIONAL SURVEY REVEALS LARGE PROPORTION OF ASYMPTOMATIC CARRIERS: CHALLENGES FOR MALARIA CONTROL IN THE REPUBLIC OF GUINEA

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In malaria endemic countries asymptomatic carriers represent a significant proportion of malaria positive individuals, harboring parasites and often not seeking treatment. Médecins Sans Frontières Switzerland has been working in Guéckédou Prefecture, the Republic of Guinea, since 2010 through a network of village health workers (VHWs), testing patients with malaria rapid diagnostic tests (RDT) and treating when positive to reduce malaria related morbidity and mortality. This two-stage, cross sectional, cluster randomized, malaria prevalence survey with PPS sampling was conducted during the dry season, February 2012. Data was collected from all randomly selected individuals who agreed to participate in the surveys, regardless of symptoms. Each participant was asked about history of malaria and fever, a short physical exam was conducted, thick and thin blood smears were made and participants were tested for malaria with an

HRPII RDT. 6,723 individuals participated in the survey. The prevalence of malaria, patients with a positive RDT, was 51.6% (95%CI: 49.2-54.1). Of those patients, 80.6% (3045/3793) were asymptomatic (non febrile with a positive RDT); there was no difference when stratified by age (P>.05). Microscopic examination of blood slides detected parasites in 82.3% (2509/3045) of asymptomatic participants. Children 5-14 years of age had the highest proportion of asymptomatic *parasitemia*, 86.9%, followed by 81.2% of children <5 years of age, and 70.8% of those ≥15 years. There was no difference in the distribution of asymptomatic *parasitemia* by gender (P=.06). The large proportion of asymptomatic carriers, a significant reservoir of infection, poses a major obstacle to malaria control programs in hyperendemic settings. Presumptive treatment strategies should be considered as a tool for malaria control. Additionally, to target this reservoir and reduce transmission, malaria diagnostic algorithms should be developed, taking into consideration the proportion of asymptomatic individuals, which lay people, including VHWs, can use.

210

PREGNANT WOMEN AND INFANTS AS SENTINEL POPULATIONS TO MONITOR PREVALENCE OF MALARIA PARASITEMIA AND TRACK IMPACT OF INTERVENTION SCALE-UP

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As malaria control interventions are intensively scaled-up, rational approaches are needed for monitoring impact over time. One proposed surveillance system approach includes ongoing malaria testing for pregnant women and young children at time of routine visits for antenatal care (ANC) and immunization services at reproductive and child health (RCH) clinics.We used a HRP-2 based malaria rapid diagnostic test (mRDT) for malaria testing to test pregnant women at time of first ANC visit and infants at time of measles immunization (age 9-12 months). This strategy was implemented in Mwanza, Mara and Kagera regions of Tanzania as a pilot to assess whether malaria screening at RCH clinics could serve as a practical approach for longitudinal surveillance of malaria prevalence. Test positivity rates (number mRDT positive/number tested) were calculated. Monthly variation in prevalence was assessed. All participants who tested positive were treated as per national guidelines. A total of 54 RCH facilities were selected between December 2012 and March 2013. The average reporting rate for RCH facilities was 85% (range 25-100%). A total of 18,911 pregnant women attended first ANC and 6,926 infants attended measles vaccination, with 52.9% and 72.6% tested with mRDT, respectively. The overall prevalence of malaria parasitaemia among pregnant women and infants was 12.2% (95% confidence interval [CI] 11.5-12.8) and 10.1% (95% CI 9.3-11.0), respectively. Variation in prevalence ranged from 10.3% in December 2012 to 14.1% in February 2013 for pregnant women and 4.4% in December 2012 to 13.6% in February 2013 among infants. Routine malaria testing of these accessible populations may offer a practical strategy for routine continuous surveillance for tracking progress of malaria control over time. Given that only a little over half of pregnant women were tested at first ANC visit, further research and efforts are needed to examine and address barriers to testing among pregnant women.

211

NATIONWIDE HEALTH FACILITY SURVEY OF SEVERE MALARIA CASE MANAGEMENT - MALAWI, 2012

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Malaria is responsible for 40% of the inpatient admissions of children under five in Malawi. The case fatality rate for severe malaria is estimated to be 10-20%. Although there have been numerous studies assessing the management of uncomplicated malaria in sub-Saharan Africa, there is little information about the quality of severe malaria case management. We systematically sampled 36 health facilities from a list of all public or mission hospitals in Malawi that admit patients with malaria, and conducted a cross-sectional survey on the quality of case management, during June-August, 2012. At each facility, we spent two days interviewing the hospital leadership and reviewing charts of suspected malaria cases to assess the overall quality of care received by patients. Charts were randomly selected from all patients who received an admission diagnosis of malaria or antimalarial treatment during October 2011 (low season) or April 2012 (high season). Univariate analysis of health facility and patient characteristics are presented. We conducted interviews at 36 facilities and reviewed 1252 inpatient records. Patients were aged 0-86, with a mean of 14 years, and 45% were female. Although only 42% of patients were given an admission diagnosis of severe malaria, 76% received intravenous quinine and <1% received artesunate. 65% of patients had parasitologic confirmation of their diagnosis on admission. On the day of the survey, 92% of the hospitals had quinine and one had artesunate available for treatment of severe malaria, with 26% of facilities noting at least one stock-out of all treatments for severe malaria within the prior three months. Rapid diagnostic tests were available at 97% of facilities, but out of stock at least once in the prior three months in 44% of facilities. Microscopy supplies were out of stock at 11% of facilities on the day of the study and 22% of facilities in the prior three months. Insufficient confirmation of malaria with laboratory diagnostics and a lack of stable supplies are two obstacles in providing optimal malaria care in inpatient settings in Malawi.

212

NATIONWIDE SURVEY OF HEALTHWORKER KNOWLEDGE OF SEVERE MALARIA CASE MANAGEMENT - MALAWI 2012

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Malaria is responsible for 40% of all hospitalizations of children <5 years in Malawi. There are limited data assessing healthworker (HW) knowledge and management of severe malaria in inpatient settings. We performed a cross-sectional survey of facilities that provide inpatient malaria care. We systematically sampled 36 facilities from a list of all public or mission hospitals in the country that admit patients with malaria; three nurses and three clinicians were randomly selected from each. HWs were interviewed about their education, training, and supervision, and tested on their malaria-related knowledge using short answer questions and sample cases. Univariate analysis of HW characteristics and responses were performed. We recruited 200 HWs from 36 facilities (106 nurses and 94

clinicians). The median age was 33 years, median years of experience was eight, and 55% were female. On the job malaria training was reported by 57% of HWs, primarily on the use of rapid diagnostic tests (RDTs). Only 5% noted they had been supervised on a malaria-related task in the prior six months, and 32% reported receiving any supervision in the prior six months. Slightly more than half (57%) of HWs were able to name at least three signs of severe malaria, 74% knew the correct treatment for a two year old with severe malaria, 72% knew the correct treatment for a pregnant female with uncomplicated malaria and 74% knew how to treat a person with fever and a negative RDT. Out of eight malaria knowledge questions, the mean number answered correctly was 5.6 (range: 2-8). HWs described difficulty with availability of treatment (58%), availability of diagnostic supplies (32%), and knowledge deficits (30%) as their main obstacles in providing malaria care. Most HWs at inpatient facilities were able to correctly state the appropriate treatment for malaria, although recognition of signs of severe disease was low. Increasing the frequency of supervision and maintaining adequate supplies for malaria diagnosis and treatment would further support the work that HWs are doing in Malawi to combat severe morbidity and mortality from malaria.

213

EVALUATION OF MALARIA CASE MANAGEMENT AND BURDEN THROUGH A NATIONAL HEALTH FACILITY SURVEY IN HAITI, 2012

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Data on malaria burden and the case management of malaria in Haiti are limited. Recent malaria guidelines for Haiti recommend dual therapy with chloroquine and primaquine for the treatment of uncomplicated malaria. In December 2012, during the primary rainy season, we conducted a nationally representative cross-sectional survey of health facilities (HFs) to determine the proportion of febrile outpatients positive for malaria and the quality of malaria case management before scale-up of diagnostics and case management training. Among Haiti's 833 HFs, 30 were selected randomly for a 2-day evaluation. Selection probability was proportional to the number of HFs per department. On day 1, HFs' material and human resources were inventoried. On day 2, all outpatients were screened for fever or history of fever; those with no severe symptoms were enrolled. HF providers evaluated and treated patients, and diagnostic tests ordered and treatment decisions were recorded. Diagnostic test results were collected from HF labs. Blood smears were obtained for gold-standard evaluation by Haiti's reference laboratory. Diagnostic capacity, defined as consistent electricity and microscopy supplies *or* presence of approved malaria rapid diagnostic tests (mRDTs), was adequate in 11 (37%) HFs. Of 115 providers, 53 (46%) had received training on malaria case management. Among 459 outpatients screened, 257 (56%) had fever or history of fever and 193 (75%) were eligible for participation, of whom 153 (80%) were enrolled. Among 39 patients with a diagnostic test result available by the end of day 2, 11 (28%) patients tested positive either by smear or RDT. Of these 11 patients, 6 (55%) were, appropriately, treated with an antimalarial. Twenty-seven (95%) of the 28 patients testing negative were, appropriately, not treated with an antimalarial, and of 114 patients without malaria diagnostic test results available, 35 (31%) were treated with an antimalarial. In total, 42 patients were treated with any antimalarial, and only 1 (2%) of these was treated according to Haiti's guidelines. Of 138 gold standard smears available from enrolled patients, none were positive. Malaria is an uncommon cause of fever in Haitian outpatients, and limited lab diagnostic capacity contributes to overdiagnosis. Planned scale-up of diagnostics and provider training on new guidelines may improve malaria diagnosis and treatment in Haiti.

214

ADDRESSING DATA HETEROGENEITY AND LONG-TERM STORAGE: THE WWARN EXPERIENCE

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The diversity of study designs and analytical methods is a major challenge when data from different studies must be compared to establish a global epidemiological surveillance system for antimalarial drug efficacy. WWARN has established a comprehensive database from which standardised estimates of antimalarial efficacy can be derived and monitored over time from diverse geographical regions and from which pooled analysis can be initiated. The WWARN database incorporates key determinants for the clinical response with *in vitro*, molecular and pharmacokinetic parameters integrating relevant data on host, drug and parasite factors. Study meta-data (location, methodology, inclusion and exclusion criteria) are systematically captured in a standardised manner. Individual patient and sample data are processed according to published data management and statistical analysis plans using specific innovative informatics tools and stored in a mySQL data repository. Data from 239 unique publications and 122 unpublished studies are present in the repository comprising 83,000 patients from clinical trials, 22,000 patients with molecular samples, 6147 with pharmacokinetic samples and 1770 *in vitro* isolates. Study years range from 1990 to 2013 with 48% of studies conducted after the year 2005. Geographically, 24% of studies were conducted in Asia, 65% in Africa and 3% in Latin America. This is the largest consolidated individual patient database assembled to date, a demonstration of the changing spirit of the malaria community to engage in data sharing. In collaboration with data contributors, WWARN has demonstrated that pooling these data sets can now provide the statistical power to test scientific hypotheses. Outcomes of pooled analyses examining dosing of ACTs, linkage of candidate molecular markers of resistance and clinical output, exploring PK variation in specific populations and testing the effect of host and parasite factors affecting parasite clearance, are in progress.

215

A SYSTEMS BIOLOGY APPROACH FOR THE DISCOVERY OF VACCINE/DRUG TARGETS IN *PLASMODIUM FALCIPARUM* USING LONG-LIVED MEROZOITES

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Malaria caused by *Plasmodium falciparum* causes several hundred million cases of clinical disease and nearly 1 million deaths each year. Passive transfer of hyper-immune human IgG from an endemic area protects children against clinical disease. Efforts to replicate this clinical immunity using blood stage recombinant protein vaccines have not been successful. Thus new discovery efforts are important. To this end, we are using a systems biology approach to identify the biological basis for a gamma irradiated parasite line to have a long-lived invasive merozoite phenotype. Cell-sieve purified long-lived merozoites significantly retain their capacity to invade RBCs at approximately 3 to 5 times that of the parent line. Analysis of the genomes has identified several SNPs leading to a stop codon in the genome of the irradiated parasite line. Using microarrays, an

overall comparison of the transcriptomes of schizonts (2 - 4 nuclei) versus purified merozoites identified early Schizont with >1400 transcripts with a 2-fold difference as compared to merozoites with only 4 transcripts. In parallel using a label-free quantitative proteomic approach (LC/MSE or LC/HDMSE), long-lived merozoites appear to have higher protein abundance after normalization to a set of 5 house-keeping proteins. Furthermore, the relative molar concentration of merozoite proteins has been determined for approximately 1300 qualified proteins. Using this information, a reconstruction of the merozoite is underway. Finally, using TransOmics™ analysis of two biological replicates evaluated by LC/MSE or LC/HDMSE, a total of 446 and 1196 proteins were identified as significantly different in protein abundance (p-value < 0.05) using Principle Component Analysis, respectively. Possibly of greater biological significance, a total 212 proteins were shared between the two groups, including 25% proteins currently identified as of unknown function. Taken all together our findings provide a unique opportunity to improve our understanding of merozoite invasion of RBCs which holds promise to aid in the discovery of new vaccine/drug targets.

216

PRE-CLINICAL EVALUATION OF NEW CHIMERIC PRE-ERYTHROCYTIC *PLASMODIUM VIVAX* ANTIGEN BASED ON CIRCUMSPOROZOITE PROTEIN

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Plasmodium vivax circumsporozoite (PvCS) protein is a major sporozoite surface antigen involved in parasite invasion to the liver cell, currently being considered as vaccine candidate. PvCS contains a dimorphic central repetitive immune-dominant domain flanked by conserved regions that contain functional domains. We have developed a chimeric 137-mer synthetic polypeptide (PvCS-NRC) that includes the conserved region I and region II-plus and the two natural repeat variants regions known as VK210 and VK247. Antigenicity studies indicated that the chimeric peptide is recognized by high proportion (60-70%) of the residents of malaria-endemic areas. Additionally, the immunogenicity of this chimeric antigen formulated either in Alum or GLA-SE adjuvants was assessed in C3H, CB6F1 and ICR mice; and a formulation of the polypeptide in Montanide ISA 51 was also tested in a group of C3H mice. Peptides formulated in both GLA-SE or Montanide ISA 51 adjuvants produced stronger antibody responses than the Alum formulation. Sera from immunized mice as well as antigen-specific affinity purified human IgG reacted with sporozoites preparations in immunofluorescence and Western blot assays, and displayed *in vitro* inhibition of sporozoite invasion (ISI) into hepatoma cells. Further evaluation of this vaccine construct is currently being conducted in Aotus monkeys in order to assess the humoral and cellular immune responses elicited by the vaccine candidate as well as its protective efficacy against parasite challenge. Results of primate studies will be presented.

217

IMMUNOGENICITY OF RECOMBINANT PROTEINS BASED ON THE DIFFERENT ALLELIC FORMS OF THE CIRCUMSPOROZOITE ANTIGEN OF *PLASMODIUM VIVAX* AIMING AT THE DEVELOPMENT OF A UNIVERSAL VACCINE AGAINST MALARIA

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Plasmodium vivax is the second most prevalent and most widespread species causing malaria in the world. Recent data estimated 132-391 million cases annually. The relative inefficiency of the measures currently used for control demands the development of new strategies for prevention such as vaccines, new drugs and insecticides. In past the 15 years, studies aimed at the development of a recombinant vaccine against the human malaria caused by the deadly parasite *Plasmodium falciparum* were based on the circumsporozoite protein (CSP). A recombinant protein expressed in yeast containing the C-terminal and part of the repeat domains of *P. falciparum* CSP in fusion with the S antigen of hepatitis B virus was used in phase III trials in African children and reported 50% efficacy. Based on these studies, we aimed at the generation of recombinant proteins in the yeast Pichia pastoris representing each of the three allelic forms of *P. vivax* CSP and fourth recombinant protein containing epitopes representing all three allelic forms in fusion in the same polypeptide. Similar to the *P. falciparum* vaccine, our recombinant proteins contained the C-terminal and the repeats domain of *P. vivax* CSP. These antigens were successfully expressed in large scale as soluble secreted proteins. After purification, they were used for experimental immunization of C57Bl/6 mice in a vaccine formulation containing also the adjuvant Poly(I:C). Immunization with any of these formulations elicited high antibodies titers (IgG) reacting with all three different allelic variants of *P. vivax* CSP. The antibodies targeted both, the C-terminal and the repeat domains of *P. vivax* CSP. Our results confirm that it is possible to elicit immunity to all three different allelic forms of *P. vivax* CSP using soluble recombinant proteins expressed from P. pastoris. We consider that these recombinant proteins are good candidates for clinical trials aiming at the development of a universal vaccine against *P. vivax* malaria (PCT/US2013/031663).

218

IMMUNOGENICITY AND PROTECTIVE EFFICACY OF MVA MALARIA VACCINE WHICH CO-EXPRESSES THE CIRCUMSPOROZOITE PROTEIN AND IL-15 IN A MOUSE MODEL OF MALARIA

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Recently, testing of the promising RTS,S vaccine in phase III clinical trials showed that this vaccine induces moderate protection against malaria in young children but the longevity of the protective response was disappointing. Thus, the search for novel strategies to deliver malaria vaccines that would induce long lasting protective immunity continues. We evaluated using *Plasmodium yoelii* 17XNL (PyNL), a non-lethal mouse model of malaria, a recombinant MVA vaccine which co-expresses the circumsporozoite protein (CSP) and the immune stimulator cytokine IL-15 (PyNL MVA-CSP/IL-15). We vaccinated C57BL/6 mice with Py NL MVA-CSP+/-IL-15 constructs at $1x10^7$ pfu/mouse/sc. Another group of mice was vaccinated with MVA-vector only as an MVA control group. One month later vaccinated and control mice were intravenously challenged with 100 Py NL SPZ. After SPZ challenge, *parasitemia*s were monitored

by blood-films taken at 3 day intervals for 25-30 days post-challenge. Mice were tested for: i) *parasitemia*, ii) anti-rCSP antibodies in ELISA using recombinant Py CSP as antigen, iii) liver parasite burden and iv) cytokines production. Results showed: i) significant reductions in *parasitemia*s in MVA-CSP/IL-15 vaccinated mice when compared to non-vaccinated mice, ii) mice vaccinated with MVA-CSP plus IL-15 had significantly reduced percent *parasitemia*s when compared to mice vaccinated with MVA-CSP without IL-15, iii) livers of mice vaccinated with MVA-CSP/IL-15 had a decreased number of parasites than control non vaccinated/SPZ infected mice, iv) MVA-CSP/IL-15 vaccinated mice produced antibodies to Py NL rCSP protein, and v) IL-6 and Eotaxin cytokines were present in the sera of MVA-CSP/IL-15 vaccinated mice but absent in the mice vaccinated with MVA-vector control group. Overall, our results suggest that Py NL MVA-CSP/IL-15 vaccine is immunogenic and partially protective and should be considered for future testing as a malaria vaccine.

219

MANUFACTURE AND *IN VIVO* TESTING OF TWO *PLASMODIUM FALCIPARUM* BLOOD STAGE PARASITE BANKS

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Controlled human malaria infection (CHMI) studies are assuming increasing importance in the development of new drugs and vaccines for malaria. Most studies are undertaken by sporozoite-induced infection, either by the bite of infected *Anopheles* mosquitos or by injection of thawed cryopreserved sporozoites. An alternative approach is to induce blood stage infection by intravenous injection of thawed cryopreserved *Plasmodium*-infected human erythrocytes. In recent times so-called induced blood stage malaria (IBSM) has been undertaken using a bank of *P. falciparum* of the 3D7 strain that had been collected in 1994 from two volunteers who had been deliberately infected with this strain by bites of infected mosquitos. The rationale for making available alternative banks for IBSM include the consideration that this malaria parasite stock will become depleted, that it is desirable that banks be manufactured according to modern cGMP principles in a controlled and regulated environment with full regulatory and ethical review, and that the availability of parasite strains of alternate genotypes and drug sensitivity would be desirable for testing of strain-transcending immunity and drug efficacy. Here we report the cGMP manufacture of a cell bank of the 3D7 strain of *P. falciparum* using modern biotechnological principles, and the collection of a bank ex vivo from a patient with naturally acquired *P. falciparum* malaria. Both parasite banks were screened and tested to rule out the presence of contaminating pathogens and then used in separate Phase I safety and infectivity studies. In each study, two volunteers were inoculated with infected erythrocytes from these banks. The characteristics of these banks will be described and the results of the *in vivo* Phase I studies will be presented.

220

SAFETY AND TOLERABILITY OF PFSPZ CHALLENGE TO VOLUNTEERS TAKING CHLOROQUINE CHEMOPROPHYLAXIS (PFSPZ-CVAC) AND PROTECTIVE EFFICACY OF PFSPZ-CVAC AGAINST CONTROLLED HUMAN MALARIA INFECTION

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Volunteers taking chloroquine ChemoProphylaxis who are immunized 3 times by the bites of 10-15 *Plasmodium falciparum* (Pf) Sporozoites-infected mosquitoes (CPS) develop complete, long-lasting protection against homologous Pf controlled human malaria infection (CHMI); exposure to 5 infected mosquitoes is less protective. Administration by the bites of mosquitoes makes this method unsuitable as a practical vaccine. To administer Pf sporozoites (PfSPZ) by needle and syringe, we have produced infectious aseptic, purified, vialed, cryopreserved PfSPZ (PfSPZ Challenge) that infect volunteers by needle and syringe inoculation. In this study we determined the safety and tolerability of intradermal (ID) administration of PfSPZ Challenge to volunteers taking weekly chloroquine chemoprophylaxis (PfSPZ-CVac), and assessed the protective efficacy of PfSPZ-CVac against homologous Pf CHMI by mosquito bites. Thirty healthy malaria-naive volunteers were enrolled in a double blind, placebo-controlled trial. All received weekly chloroquine. Twenty volunteers received 3 immunizations with 75,000 PfSPZ at 4-week intervals. The 10 control subjects received 3 injections of normal saline. Ten immunized subjects and 5 controls had homologous Pf CHMI by mosquito bites 60 days after last immunization (32 days after last dose of chloroquine). PfSPZ-CVac immunizations did not cause acute systemic allergic reactions or local adverse events (AEs), and there were minimal related AEs. Two out of 10 PfSPZ-CVac subjects remained thick smear negative through day 21 after CHMI, and one was parasite negative by PCR. One PfSPZ-CVac immunized subject experienced an episode of myocarditis (serious adverse event) following treatment for malaria. Three doses of 75,000 PfSPZ were safe and well tolerated. However, this regimen, which is not comparable to 5 mosquito bites in terms of infectivity, was not adequate for immunization. In subsequent studies regimens of PfSPZ Challenge with infection rates comparable to the bites of 10-15 PfSPZ-infected mosquitoes will be assessed.

221

VACCINE FORMULATIONS AGAINST *PLASMODIUM VIVAX* MALARIA USING COMBINATIONS OF MEROZOITE RECOMBINANT ANTIGENS

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Malaria is one of the priorities of global research in the area of vaccine development. Due to the malaria parasite antigenic variation, an effective vaccine formulation should elicit protective immune responses to a combination of immunodominant and sub-dominant antigens of the parasite. Based on that, this study aimed at characterizing of the immune responses induced by immunization of mice with formulations containing the immunodominant Merozoite Surface Protein 1 (MSP-1) antigen and the sub-dominants Apical Membrane Antigen 1 (AMA-1) and Merozoite Surface Protein 3β (MSP-3β) of *Plasmodium vivax*. The recombinant proteins were produced in bacteria ($MSP1_{19}$ and MSP3β) or *Pichia pastoris* (AMA-1 and $AMA1_{66}$-$MSP1_{19}$). The immunogenicity of the different

formulations was evaluated in C57BL/6 and BALB/c mice administered in the presence of the adjuvant Poly (I:C). IgG antibody titers were estimated by ELISA and cell-mediated immune responses by T-cell proliferation and IFN-γ production. C57BL/6 mice immunized with the recombinant antigen combinations displayed high antibody titers (>log10[4]) similar to mice inject with each antigen alone. In contrast, BALB/c mice immunized with this same vaccine formulation had lower antibody titers to $MSP1_{19}$ and AMA-1 compared to mice inject with each antigen alone. Interestingly, this interference was not observed in mice immunized with the chimeric protein $AMA1_{66}$-$MSP1_{19}$. CD4(+) T-cells were able to proliferate and produce IFN-γ against AMA-1. Together, in general, our data support the combination of antigens as a possible strategy for vaccination against malaria.

222

PRECLINICAL PRIORITIZATION OF BLOOD-STAGE MALARIA VACCINE CANDIDATES OF *PLASMODIUM FALCIPARUM* USING WHEAT GERM CELL-FREE SYSTEM

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Malaria kills approximately one million people in the tropics every year. Emergence of drug resistant parasites interferes with malaria control efforts and highlights the critical need for effective vaccines. However, not a single licensed vaccine has been developed to date. In order to exploit the Malaria Genome Database information for the discovery of novel vaccine candidate molecules, we here utilized a functional approach based on the wheat germ cell-free system. We bioinformatically selected 117 putative schizont/merozoite stage-specific molecules, and synthesized all of the recombinant proteins using the wheat germ cell-free system in antigen scale. We then raised specific antibodies against these recombinant proteins in rabbits. When we screened all the antibodies, we found that the antibodies against several molecules had the capacity to limit invasion and/or growth of *Plasmodium falciparum* 3D7 parasites *in vitro*. Among them, we found that novel candidates GAMA, MSPDBL1, RALP1, and several other hypothetical proteins ranked highly in the *in vitro* growth inhibition assay, in addition to known vaccine candidates such as Rh5, EBA175, AMA1, and MSP1. In order to evaluate the combined effects of antibodies on invasion, we then tested the combination of antibodies against GAMA (involved in sialic acid (SA) independent invasion pathway), and EBA175 (involved in SA dependent pathway), as an example. This anti-GAMA and anti-EBA175 combination exhibited a significantly higher level of invasion inhibition, supporting the rationale that targeting of both SA-dependent and SA-independent ligands/pathways is better than targeting either alone. Results presented in this study validate the use of a combination of these two ligands as a potential vaccine that would have broad activity against *P. falciparum*. Our data clearly shows that this functional approach is a reliable post-genome strategy not only for the identification and the prioritization of novel blood-stage malaria vaccine candidates, but also for the discovery of novel antigen combinations for potential blood-stage vaccines.

223

ANTIBODIES AGAINST CSP AND MSP5 PREDICT IRRADIATED SPOROZOITE VACCINE MEDIATED PROTECTION AGAINST MALARIA

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A clinical trial testing the immunogenicity and efficacy of purified, irradiated, cryopreserved sporozoites (PfSPZ Vaccine from Sanaria) administered by intravenous injection resulted in 13 individuals that were protected against controlled human malaria infection by mosquito bite and 19 that were not protected. In the highest dose group 6 out of 6 individuals were protected and the next lower dose group had 6 out of 9 protected. The specimens from this trial provide an opportunity to determine immune parameters associated with the vaccine mediated protective response. We constructed a microarray containing 4,528 *Plasmodium falciparum* (Pf) protein features representing 50% of the parasite proteome and probed it with the specimens from this trial. 100% of the protected subjects and 23% of the unprotected had significant antibody levels against the Pf circumsporozoite protein (CSP). 77% of the protected and 0% of the unprotected had antibodies against MSP5. CSP and MSP5 used together in a multiplex serodiagnostic assay predicted protection from malaria with 92% sensitivity and 89% specificity. These results suggest that antibody responses to CSP and MSP5 are an accurate biomarker of PfSPZ vaccine mediated protection against malaria. CSP is already a well-recognized target antigen for vaccine development and these results suggest that MSP5 should also be investigated as a vaccine candidate. We are in the process of constructing the complete Pf proteome array which will contain 9000 protein features representing 100% of the Pf proteome. When this full array is probed with the same specimens we anticipate discovering additional antibody biomarkers associated with protection and novel vaccine candidates.

224

UTILITY OF SERUM REPLACEMENT FOR GAMETOCYTE CULTURE OF *PLASMODIUM FALCIPARUM* TO PERFORM THE STANDARD MEMBRANE-FEEDING ASSAY

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There has been a renewed interest in the development of Transmission-blocking (TB) vaccines against *Plasmodium falciparum* malaria. The standard membrane-feeding assay (SMFA) is one of the functional assays by which the TB activity of test antibodies is evaluated using cultured *P. falciparum* gametocytes and *Anopheles* mosquitoes in pre-clinical and clinical studies. While the SMFA provides useful information, there are several technical difficulties in performing the assay. One of the major issues is to obtain a "suitable" human serum batch which supports gametocyte culture. Researchers usually make pools of sera from multiple donors for gametocyte culture. However, some serum pools produce few or no gametocytes, and some batches produce gametocytes but result in poor oocyst development in mosquitoes. Bovine serum albumin (e.g., AlbuMax) has been used successfully as a substitute for human serum for asexual stage culture, though AlbuMax has not reported effective for sexual stage culture using. Human serum albumin (HSA) and Serum Replacement (SR) are becoming widely used for embryonic stem cell culture. Therefore, in this study we tried to maintain gametocyte cultures using HSA (from Irvine Scientific) or a SR (from Sigma), instead of 10%

human serum. The HSA medium did not induce any gametocytes. On the other hand, the medium containing SR (5% of SR and 5% of human serum) induced stage V gametocytes *in vitro*. We fed the gametocyte culture to A. *stephensi* mosquitoes and confirmed that the gametocytes could convert to oocysts in the mosquitoes 8 days after feeding. By using the SR we could reduce consumption of "suitable" serum by half. We continue to evaluate other HSA and SR, and are also trying other reagents to achieve a serum-free gametocyte culture which can produce oocysts in mosquitoes.

225

FINE MAPPING THE B-CELL EPITOPES ON *PLASMODIUM FALCIPARUM* CIRCUMSPOROZOITE PROTEIN RECOGNIZED BY INDIVIDUALS LIVING IN A MALARIA ENDEMIC AREA

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To assist design a superior *Plasmodium falciparum* circumsporozoite protein (*PfCSP*) based vaccine, we are systematically mapping the human B-cell epitopes on the entire *PfCSP*. This approach is based on reactivity of recombinant *PfCSP* constructs encoding for the full length (Q_{21}-K_{373}), NH_2-terminal (G_{27}-R_{106}) and carboxyl terminal domains (117 AAs) against a pool of 10 plasma samples collected from clinically immune adults in Ghana. A pool of 10 plasma samples from United States blood donors served as controls. The synthetic peptides used in this study included the 20-mer overlapping peptides encompassing the NH_2 terminal (Q_{21}-K_{95}) and repeat region $(NANP)_6$. In addition, we have synthesized 10 overlapping peptides representing the NH_2-terminal (Q_{21}-P_{102}) region that were designed based on the prediction using the Abdesigner software (a tool that analyzes the peptide based on immunogenicity, uniqueness and conservation score). The pooled Ghanaian plasma had high level of ELISA IgG titers against *rPfCSP* full length, and recombinant and synthetic *PfCSP* NH_2-terminal and $(NANP)_6$ repeats. Thus, apart from the repeat domain, naturally occurring antibodies are present in immune adults against the NH_2-terminal region of *PfCSP*. Among the six NH_2-terminal (20-mer) peptides, the highest ELISA IgG reactivity was localized against the three peptides representing amino acids L_{51}-K_{95}. Further finer epitope mapping using the 10-mer epitopes revealed that the reactivity was against the 4 of 10 peptides that represented the amino acids N_{68}-N_{83}. The minimal NH_2-term B-cell epitope(s) are being mapped using the overlapping 5-mer peptides. Similar studies are underway to map the minimal B-cell epitope(s) on the carboxyl-terminal region. Next, we will compare the reactivity of *PfCSP* domains and the minimal B-cell epitopes against sera from young children under the age of 5 and immune adults. Finally, based on ELISA reactivity, an immunological score will be ascribed to the *PfCSP* domains and B-cell epitopes identified in this study. We think that the extensive B-cell mapping for the entire *PfCSP* may help guide to develop a superior malaria vaccine.

226

ANTIBODY AGAINST THE CIRCUMSPOROZOITE PROTEIN INDUCED BY ADENOVIRUS 5-VECTORED *PLASMODIUM FALCIPARUM* VACCINE IN ADULTS

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Antibody (Ab) against the circumsporozoite protein (CSP) likely mediates the partial protective immunity to malaria induced by the CSP-based RTS,S vaccine and may also contribute to the high-grade immunity induced by radiation-attenuated *Plasmodium falciparum* sporozoites. NMRC-M3V-Ad-PfCA (AdCA), a recombinant human adenovector vaccine (human serotype 5) expressing CSP and a second malaria antigen, apical membrane antigen-1 (AMA1), when primed with DNA expressing the same antigens, induced robust antigen-specific T-cell responses and provided sterile protection against controlled human malaria infection (CHMI) in 27% of recipients. The protection was associated with interferon-gamma secreting CD8+ T cells. Ab responses induced by AdCA, however, were low. To compare the humoral immunogenicity of AdCA with published data on RTS,S in a larger sample, we conducted ELISAs utilizing the CSP repeat sequence, NANP as capture antigen and measured the amount of anti-CSP Ab in micrograms/mL in serum from 56 research subjects receiving the AdCA vaccine (or the CSP component, AdC). Compared with the anti-CSP Abs induced by RTS,S (reported geomean 64.4 - 143.5 mg/mL), the levels obtained with our adenovector fall short (0 - 12.9 ug/mL; geomean 1.67 mg/mL), and are well below the threshold of 20 mg/mL above which about 50% of research subjects immunized with RTS,S are protected. Interestingly, anti-CSP Ab in the four study subjects with significant pre-existing neutralizing antibodies to the Ad5 backbone were within the lowest range (0 - 1.2 mg/mL). Combination vaccine approaches with protein-based vaccines may be required to induce both cellular and humoral immunity.

227

ANTIBODIES TO SCHIZONT EGRESS ANTIGEN-1 (PFSEA-1) PREDICT RESISTANCE TO SEVERE *FALCIPARUM* MALARIA IN CHILDREN

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Our goal is to discover novel vaccine candidates for pediatric *falciparum* malaria by identifying the parasite targets of naturally acquired protective human antibodies. We applied our differential, whole proteome screening method using plasma and epidemiologic data from a birth cohort of children living in Tanzania to identify novel *Plasmodium falciparum*

antigens associated with resistance in 2 yr old children. We pooled plasma from 12 resistant (RP) and 11 susceptible (SP) 2 yr old children and performed differential screening experiments on a *P. falciparum* 3D7 strain blood stage cDNA library. We identified Schizont Egress Antigen-1 (PfSEA-1), a 244-kDa-parasite antigen that was uniquely recognized by antibodies in RP but not SP. We have expressed and purified the immuno-relevant region in *E. coli* and designated this protein rPfSEA-1A. In an initial validation ELISA using a completely independent selection of resistant and susceptible individuals from our Tanzanian birth cohort, IgG antibody recognition of rPfSEA-1A was 4.4 fold higher in RP (n=11) than in SP (n=14, $P < 0.0002$), yet did not differ for other malarial proteins (RAMA, MSP-1, MSP-3 LSA-N, LSA-C, or controls. We next measured anti-PfSEA-1A antibodies in all two yr olds in our birth cohort and related these responses to malaria outcomes. Children in our cohort experienced a dramatically increased incidence of severe malaria during periods with undetectable anti-PfSEA-1 antibody levels (45 cases/23,806 child weeks) compared to periods with detectable antibody levels (0 cases/1,688 child weeks). In multivariate, repeated measures models, anti-PfSEA-1 antibodies were associated with significantly lower risk of severe malaria even after adjusting for age, hemoglobin phenotype and prior *parasitemia* (adjusted OR 4.4; Type III fixed effects $P < 0.03$). These results support the development of PfSEA-1 as a novel vaccine candidate for pediatric *falciparum* malaria.

228

EFFICIENT INFECTION OF RHESUS MACAQUES WITH PURIFIED, CRYOPRESERVED *PLASMODIUM KNOWLESI* SPOROZOITES

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Intravenous (IV) injection of Sanaria's radiation-attenuated, purified, cryopreserved PfSPZ Vaccine has recently been shown in volunteers to induce high level protection against controlled human malaria infection (CHMI). Despite this success and the promise of a whole PfSPZ vaccine, we cannot definitively determine in humans the precise immunological responses that contribute to protection. Moreover T cell responses in the blood and in the critically important liver often do not correlate. *Plasmodium knowlesi* (Pk) represents a relatively rare instance in which the same malaria parasite infects humans and non-human primates (NHPs) in nature, and has served as a relevant NHP model for Pf malaria for years. Sanaria has therefore used the methods developed for PfSPZ to manufacture purified, vialed, cryopreserved PkSPZ. We show that these PkSPZ can infect human hepatocytes *in vitro*. In a dose response study to determine the lowest dose of PkSPZ required to infect NHPs (rhesus macaques), 500, 2,500, 12,500 and 25,000 PkSPZ were injected IV into 3 animals per group. All animals developed patent *parasitemia*. The time to patency in the three highest groups was comparable to pre-patency observed after injection of freshly-dissected PkSPZ and all animals within a group were uniformly parasitemic on days 9 (2,500), 8 (12,500) and 7 (25,000) respectively. In the 500 PkSPZ group pre-patency ranged from 9-12 days although all animals became parasitemic. Our development of cryopreserved PkSPZ that efficiently infect NHPs paves the way to establish regimens of radiation-attenuated PkSPZ and infectious PkSPZ administered with a chemoprophylactic (PkSPZ C-Vac) that protect the NHPs against controlled infections. This model can then be used to conduct in-depth analyses of immunological mechanisms and correlates of protection that cannot be substantiated in humans, particularly those in the critically important sites in the liver.

229

A MULTI-STAGE MULTI-ANTIGEN VACCINE FOR INTERRUPTING MALARIA TRANSMISSION

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Eradication of malaria would be facilitated by a potent vaccine that interrupts malaria transmission (VIMT). An ideal VIMT would prevent infection, disease, and transmission by targeting at a minimum the pre-erythrocytic (sporozoite [SPZ] and liver stages) and optimally additionally at least the sexual-mosquito stages of the life cycle. We produced two recombinant (r) pre-erythrocytic stage antigens, *Plasmodium falciparum* (Pf) CSP and rPfCelTOS. When mice were immunized with rPfCelTOS alone, rPfCSP alone, or both, mice immunized with both proteins had higher titers of antibodies against PfSPZ by IFA and activity in blocking PfSPZ invasion and development in hepatocytes (86%) than did mice immunized with rPfCSP (53%) or rPfCelTOS (20%) alone. PfCelTOS is also expressed in Pf ookinetes, and antibodies against PfCelTOS blocked transmission in mosquitoes comparable to a monoclonal antibody against Pfs25. The observation that antibodies against PfCelTOS had biological activity against pre-erythrocytic (SPZ) stages, were additive or synergistic with anti-PfCSP antibodies, and blocked transmission at the mosquito stage were unique. To further enhance VIMT effects, we produced recombinant Pf von Willebrand factor A domain-related protein (PfWARP), a highly conserved, soluble ookinete specific protein that we have shown previously to potently inhibit development of oocysts in the mosquito midgut. Our aim is to develop a combined multiple stage vaccine to prevent transmission by inhibition of development of sexual blood stage parasites and oocysts. PfCelTOS and PfCSP are also expressed in the hemocoel stage of PfSPZ and thus our strategy would also target the conversion of oocysts to infectious PfSPZ in salivary glands. We will report the biological activity of antibodies against PfCSP, PfCelTOS, and PfWARP alone and in combination against invasion and development of PfSPZ in hepatocytes and oocyst development in mosquitoes.

230

DETERMINING THE EFFECTIVENESS IMMUNOGENIC ANTIGENS OF *ECHINOCOCCUS GRANULOSUS* IN EXPERIMENTALLY INFECTED DOGS

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Cystic echinococcosis is a serious parasitic zoonosis in Peru and other developing countries. The causative agent is the tapeworm *Echinococcus granulosus*, whose adult stage takes place in the small intestine of the definitive hosts(dogs) and the development of the larval stage occurs mainly in the liver and lung of intermediate hosts such as sheep and accidentally the human being. A vaccine to protect dogs would reduce the parasite biomass by reducing the number of eggs that might infect the livestock. Therefore immune-protection was evaluated against E.granulosus using surface antigens as membrane proteins and metabolic products of excretion-secretion protoscoleces and adult worms. Membrane proteins were obtained by extraction with Triton x-114(2%) and the products of excretion/secretion(E/S) obtained by *in vitro* culture, using HAM F12 Medium supplemented with glucose(2%), glutamine(0.5%) and L-arginine(0.5%). Furthermore, the total antigen of whole adult stage was obtained by sonication. Quil A was used (50ug/ml) as the adjuvant. The antigens were administered for intranasal route. A total of 12 dogs were allocated to one of the four treatment groups: control (Quil A with HAM F12 medium), E/S (200ug protoscoleces and 200ug adult tapeworm), PM (200ug protoscoleces and 200ug adult tapeworm) and total protein

(200ug adult tapeworm). Animals received three immunizations at 15 days intervals; 15 days after the last immunization, all animals were orally challenged with 150000 live protoscoleces obtain from liver cyst recovered from naturally infected sheep. The dogs were euthanized between 49-53 days post-challenge by intravenous lethal injection. The median parasite load and presence of eggs were evaluated. Groups E/S(7250) and PM(7333) had lower median parasite load and absence of eggs compared to the control(28333) and total protein group(14000), although parasite load was no statistically significant difference. Also, increase in systemic antibody titers of IgG isotype was detected by indirect ELISA in plasma as a result of the immunization in E/S and PM groups. The results show the immunization of E/S and PM antigens, at low concentrations are a good alternative for the protection of dogs against E.granulosus and the possibility of continuing studies with these antigens, since inhibition of embryo development is critical to stop the transmission intermediate hosts and man.

231

MEDICAL TREATMENT VS. "WATCH AND WAIT" IN THE CLINICAL MANAGEMENT OF CE3B ECHINOCOCCAL CYSTS OF THE LIVER

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Despite the suggested stage-specific choice of treatment, echinococcal cysts in the transitional stage CE3b remain the most challenging ones as they are unresponsive to non-surgical treatments. We compared retrospectively ABZ vs "Watch and Wait" in patients with single hepatic CE3b cysts seen at our clinic who either received ABZ or entered the WW group at diagnosis. Primary endpoints were inactivation (CE3b to CE4) and relapse (CE4 to CE3b). Variables included sex, age at first consultation, origin, longest diameter of cyst (S <50 mm, M: 50-100 mm, L >100 mm), and treatment. The secondary endpoints were type, rate of complications (mild and severe events), correlation of cyst dimension to probability of complication. Sixty patients were enrolled 34 (57%) males 26 (43%) females, median age 43.6 (range 8-75), 23 (47% of 49 cysts we had information on) had M cysts Thirty-five patients received some course of ABZ followed by at least 24 months of WW, and were divided into 3 groups: ABZ only (17), ABZ and WW (35) and WW only (8). In these groups, 12 cysts became inactive, 18 relapsed and 30 remained unchanged. Uni- and multivariate analysis showed that ABZ treatment was positively related to inactivation (with p-val 0.001, hr 7.186 IC 2.66-19.40) More cysts on ABZ reached inactivation and in a shorter time than those on WW. None of the variables had a statistically significant correlation with relapses. As for the secondary endpoints, the confidence intervals of the treatment and the WW group overlapped and complication rates were similar. For cysts on WW, mild events in L were 3.5%, compared to 2.5% in S and M groups. L cysts' severe complication rate was 4.8% vs 1.4% of S and M. ABZ induces cyst inactivation in more patients and in a shorter time than WW. Many CE3b cysts remain unchanged over time and some CE3b cysts originate from a relapsing CE4. Three patients with L cysts underwent surgery while on WW. Expectant management may be a viable option for S and M CE3b cysts; L cysts may need surgery.

232

EXPERIMENTAL INFECTIONS OF YOUNG PIGLETS BY DUNG BEETLES INOCULATED WITH *TAENIA SOLIUM* EGGS

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Taenia solium, the porcine tapeworm, is a public health problem that can cause human teniasis as well as human neurocysticercosis. Pigs infected with, *T. solium*, can develop porcine cysticercosis which results in economic hardship for small-scale farmers in the developing world. We hypothesize that dung beetles act as mechanical vectors in the transmission of taeniasis/cysticercosis. The objective of this project was to determine whether 1-4 week old piglets may be infected by dung beetles carrying eggs of *T. solium* and whether these piglets could develop a response to subsequent infections. Twenty-four piglets were acquired (16 newborns and 8 two-months olds). Dung beetles were fed proglottids, and each piglet was fed 6 beetles. Infections were performed at the ages of 1, 2, 3, and 4 weeks. The two-month old pigs were also infected using dung beetles and served as positive controls for age. The pigs were categorized into two groups: Group A which was humanely euthanized after 8 weeks post infection, and Group B that received a second dose of beetles at 8 weeks and were humanely euthanized at 16 weeks. Thorough necropsy and evaluation were utilized to count the number of parasitic cysts in each pig. Cysts were found in 21 of the 24 infected pigs. Using a negative binomial regression analysis, no statistical difference was found between the number of cysts in animals infected once, versus animals infected twice. Overall, the animals had an average of 8 cysts, ranging from 0 to 37. No statistical difference was found between porcine age and the burden of cysts (p= 0.68 Kwallis test). This study demonstrates that dung beetles can act as mechanical vectors of *T. solium* eggs. In contrast to existing literature, our study has shown that shown that one-week old piglets are capable of developing viable cysts within their skeletal muscles. Reinfection of group B pigs did not enhance nor diminish the presence of cysts. Our study illustrates that the number of cysts after infection by beetle vectors is relatively low. It is possible and even likely that this low cyst burden within the musculature may have go unnoticed by sanitary inspectors since identification of the rare cysts required meticulous dissection of the tissue, a more detailed process than typically afforded in the food inspection process. For this reason, dung beetles may play an important role in the transmission and persistence of this parasite.

233

QUANTIFYING THE PROTEIN CONTENTS IN VESICULAR FLUID OF *TAENIA SOLIUM CYSTICERCI* FOR DIAGNOSING CYSTICERCOSIS IN HUMANS

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The World Health Organization , about neurocysticercosis(NCC) says "the most important neurological disease caused by parasites in humans" and it is endemic in Peru, with prevalence from 0.72-12%(Lima hospitals) and 8%(Tarapoto jungle). The diagnosis of NCC is made with Immunoblot tests, but are not routinely performed in Peru because of its high cost. Also, NCC surveillance and control have not been adequately implemented by the Peruvian Ministry of Health(there is no program for NCC). The Parasitic Zoonosis Laboratory of the Peruvian National Institute of Health is currently working in the development of a low cost and good quality method for diagnosing NCC. The objective of the study is to quantify the antigenic proteins in the vesicular fluid of *Taenia solium cysticerci*(LVC) for diagnosing the infection in humans, thus contributing to solve an important public health problem. This is an observational study. The fluid was obtained by dissecting muscle from pigs from endemic zones with cysticercosis, these are Huancayo, Huanuco, Ayacucho, and Ucayali, and each one was assigned an antigenic batch. The Regional Reference Laboratories from endemic zones in which the vesicular fluid was obtained, and the Parasitic Zoonosis Laboratory in the Peruvian National Institute of Health did the protein quantification. The protein concentration in each batch was determined using the Lowry technique and the standard curves for bovine serum albumin and LVC-total Ag for *T.sol*.were plotted. The electrophoresis profile of the antigens batches was obtained and the known molecular weights using Western blot were identified: 13, 14, 17, 18, 21, 23, 24, 31, 35, 42-45, 66 and 97kDa for the immunodiagnosis of human cysticercosis. Five antigens were quantified using Lowry(Huanuco, Ucayali, Huancayo-L, Ayacucho, and Huancayo-M). Total antigen contents from Ayacucho(0.78 mg/mL) and Huancayo-L(0.52mg/mL) have low protein concentrations; however

Huancayo-M(3.596mg/mL), Huanuco(0.94mg/mL), and Ucayali(0.81mg/mL) have higher protein concentrations. There are high levels of protein concentrations including LVC-total antigen for *T.solium*, but with great variability between endemic zones. The reasons for this are not known. The highest total protein antigen concentrations were found in samples from Huancayo-M, Huanuco, and Ucayali. The US.CDC considers the protein concentration of the LVC-total *T.sol.* purified antigen lies between 3-5µg/mL.

234

A LATERAL FLOW RAPID TEST USING RECOMBINANT PYRUVATE PHOSPHATE DIKINASE OF *ENTAMOEBA HISTOLYTICA* FOR DETECTION OF AMOEBIC LIVER ABSCESS

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Amoebiasis, caused by the intestinal protozoan parasite *Entamoeba histolytica*, is an important parasitic cause of death in humans; and mortality is mainly due to amoebic liver abscess (ALA). Currently, several serological methods are available to diagnose ALA, however they are based on native antigens and are not in rapid lateral flow format. Previously we have reported that *E. histolytica* pyruvate phosphate dikinase (PPDK) is a potential diagnostic marker for ALA. Here, we report the development and evaluation of a lateral flow rapid dipstick test using recombinant form of the protein (rPPDK) for the detection of specific antibodies to *E. histolytica* in human sera. It involved three steps: (I) Expression of rPPDK in *Escherichia coli* expression system followed by affinity purification using Ni-NTA resin; (II) Evaluation of the diagnostic sensitivity and specificity by western blot using sera from patients with ALA and controls (healthy individuals and other parasitic infections). Horseradish peroxidase conjugated anti-human IgG and IgG4 antibodies were used as secondary antibodies; (III) Development and evaluation of a lateral flow rapid dipstick test for detection of anti-PPDK antibodies in the serum samples. The expressed recombinant protein had an estimated molecular mass of 98 kDa. Western blots showed that the purified rPPDK probed with anti-human IgG4-HRP was immunoreactive with all patients' samples and not reactive with control sera. The purified rPPDK at 1.25 mg/ml and goat anti-mouse IgG at 0.5 mg/ml were lined on nitrocellulose membrane cards as test and control lines, respectively. After blocking and cutting into strips, the lateral flow rapid dipstick tests were evaluated with 30 ALA sera samples and 40 control sera using anti-human IgG4 conjugated with colloidal gold. The dipstick test showed 87% sensitivity and 100% specificity. Further validation studies will use larger number of serum samples, including comparison of pre and post-treatment samples. In conclusion, the lateral flow dipstick test using rPPDK showed potential utility for rapid diagnosis of amoebic liver abscess.

235

NEUROPSYCHOLOGICAL EFFECTS AND BIOMARKERS OF KONZO: A NEUROMOTOR DISEASE ASSOCIATED WITH POORLY PROCESSED CASSAVA

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Konzo is an irreversible upper-motor neuron disorder affecting children dependent on bitter cassava for food. Although the neuroepidemiology of konzo is well characterized, we report the first neuropsychological findings. Children with konzo in the Democratic Republic of Congo (mean age 8.7 years) were compared with children without konzo (mean age 9.1 years) on the Kaufman Assessment Battery for Children, second edition (KABC-II), and the Bruininks-Oseretsky Test of Motor Proficiency, second edition (BOT-2). Both groups were also compared with normative KABC measures from earlier studies in a nearby nonkonzo region. Using a Kruskal-Wallis test, children with konzo did worse on the KABC-II simultaneous processing (visual-spatial analysis) (K [1] = 8.78, P = .003) and mental processing index (MPI) (K [1] = 4.56, P = .03) than children without konzo. Both konzo and nonkonzo groups had poorer KABC sequential processing (memory) and MPI relative to the normative group from a nonkonzo region (K [2] = 75.55, P, .001). Children with konzo were lower on BOT-2 total (K [1] = 83.26, P, .001). KABC-II MPI and BOT-2 total were predictive of konzo status in a binary logistic regression model: odds ratio = 1.41, P, .013; 95% confidence interval 1.13-1.69. Motor proficiency is dramatically affected, and both children with and without konzo have impaired neurocognition compared with control children from a nonoutbreak area. This may evidence a subclinical neurocognitive form of the disease, extending the human burden of konzo with dramatic public health implications. Novel biomarkers of konzo include elevated serum levels of F2-isoprostanes suggesting that oxidative stress may play a role in the pathogenesis of the disease.

236

MALARIA IS AN UNCOMMON CAUSE OF ADULT SEPSIS IN A MESOENDEMIC REGION OF UGANDA

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Malaria is often considered a cause of adult sepsis in malaria endemic areas. However, diagnostic limitations can make distinction between malaria and other infections challenging. Therefore, we sought to determine the relative contribution of malaria to adult sepsis in a mesoendemic area of southwestern Uganda. We enrolled adult patients with sepsis at the Mbarara Regional Referral Hospital between February and May 2012. We defined sepsis as infection plus [greater than or equal to]2 of the following: axillary temperature >37.5degreesC or <35.5degreesC, heart rate >90, respiratory rate >20, or a total leukocyte count >12,000 or <4,000 cells/ul. We defined severe sepsis as sepsis plus organ dysfunction (blood lactate >4mmol/l, confusion, or a systolic blood pressure <90 mmHg). We collected sociodemographic, clinical, and laboratory data, including malaria PCR and rapid diagnostic tests, and

blood and acid fast bacteria sputum smears. We followed patients until inpatient death or discharge. Our primary outcome of interest was the cause of sepsis. We also performed multivariable logistic regression to assess predictors of mortality. We enrolled 216 participants who were 51% female with a median age of 32 years (IQR 27-43 years). Of these, 122 subjects were HIV-seropositive (56%) of whom 75 (66%) had a CD4 count <100 cells/uL. The prevalence of malaria was 3.7% (6 with *Plasmodium falciparum*, 2 with *P. vivax*). Bacteremia was identified in 41 (19%) patients. In-hospital mortality was 19% (n=42). In multivariable regression analysis, Glasgow Coma Score <9 (IRR 4.81, 95% CI 1.80-12.8) and severe sepsis (IRR, 2.07, 95% CI 1.03-4.14), but no specific diagnoses, were statistically associated with in-hospital mortality. In conclusion, malaria was an uncommon cause of adult sepsis in a regional referral hospital in southwestern Uganda. In this setting, we recommend thorough evaluation for alternate causes of disease in patients presenting with sepsis.

237

HOSPITAL-BASED SURVEILLANCE FOR ROTAVIRUS AND INTUSSUSCEPTION AMONG YOUNG CHILDREN IN BANGLADESH

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The Government of Bangladesh plans to include a rotavirus vaccine in the country's immunization program in 2014 to reduce hospitalizations and deaths due to rotavirus. The Institute of Epidemiology, Disease Control and Research and icddr,b began surveillance in June 2012 to generate geographically representative pre-vaccination baseline data for rotavirus and intussusception-related hospitalizations, and describe circulating rotavirus strains. In five tertiary hospitals throughout Bangladesh, research staff collected fresh stool samples, and demographic and clinical information from every 4th patient aged <5 years admitted with acute gastroenteritis (AGE), defined as ≥3 watery stools or ≥1 episode of forceful vomiting within a 24-hour period. We used a 20-point Ruuska-Vesikari severity scale to measure the clinical severity of patients' symptoms. Stool samples were tested for rotavirus antigens by enzyme immune assay, and 25% of the rotavirus positive specimens were selected for genotyping. Children <2 years of age hospitalized with intussusception confirmed by either surgery or radiology were listed and followed-up at home one month after discharge to ascertain outcomes. From July 2012 to February 2013, we enrolled 537 AGE patients; 71% had rotavirus antigens detected in stool; two of these children died. The majority (52%) of the confirmed rotavirus case-patients were 6-11 months of age. The proportion of AGE cases with rotavirus peaked between November 2012 through February 2013 (median 80%). Clinical severity was significantly higher (mean 13.1 vs. 12.2, $p < 0.01$) in children with compared to those without rotavirus. Among 60 strains genotyped, G1 (45%) was the most common strain followed by G12 (35%) and G9 (20%). Twelve children were diagnosed with intussusception and one died. Rotavirus is a major cause of childhood hospitalization for AGE in Bangladesh, and exhibits considerable genotypic diversity. It will be important to continue surveillance through the introduction of vaccine to estimate the reduction in rotavirus hospitalizations, describe changes in strain diversity, and to identify any increase in patients seeking care for intussusceptions which could represent adverse events.

238

ISOLATION, DETECTION AND MOLECULAR CHARACTERIZATION OF *LEPTOSPIRA SPP.* ISOLATED FROM ANIMAL AND ENVIRONMENTAL SOURCES IN MALAYSIA

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Leptospirosis is a globally important zoonotic disease caused by spirochetes of the genus *Leptospira*. In Malaysia, leptospirosis is an emerging disease and determination of the circulating serovars is essential to public health. Isolation of *Leptospira* is confirmatory for diagnosis; however, culture is insensitive and often takes months for the organisms to grow. Therefore, there is a need for rapid diagnosis and identification of *Leptospira* to guide public health interventions during outbreaks. This study aimed to develop a rapid PCR-based assay for early detection of *Leptospira* using primers that targeted the 16SrRNA gene. A second primer set targeted the *ligB* gene to differentiate pathogenic strains. Specificity and sensitivity was 100% using 12 *Leptospira* reference strains of multiple species and 10 non-leptospiral bacteria. The pathogenicity of all 12 reference strains could be differentiated using the *ligB* PCR. The limit of detection was 23.1pg DNA and 10^3 leptospires/ml in spiked urine and water. Samples from rats (n=350), dogs (n=150), cats (n=50), water and soils (n=120) were used in this study. A total of 34 isolates were confirmed as *Leptospira* genus (25 rats, 1 dog, and 8 water). Twenty-nine isolates were classified as pathogenic using the PCR while the remaining 5 were saprophytic. The genomic diversity of the 34 *Leptospira* isolates was determined using pulsed-field gel electrophoresis (PFGE) and randomly amplified polymorphic DNA (RAPD). PFGE of *Not* I-digested chromosomal DNA subtyped the 34 isolates into 11 pulse types, while RAPD produced 18 profiles. Both PFGE and RAPD were able to differentiate the zoonotic and environmental isolates. In conclusion, primers developed for the PCRs were able to successfully determine the genus and pathogenic status of the *Leptospira* strains. With its specificity and rapidity, these PCR tests are a promising tool for the early detection of *Leptospira spp.* from different sources. RAPD could be an alternative subtyping tool for *Leptospira* isolates as it is easier and could generate results more rapidly than PFGE.

239

SURGICAL NODULECTOMIES CAN HEAL IN LYMPHODEMA PATIENTS IN RESOURCE POOR SETTINGS

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Podoconiosis, a geochemical dermatosis leading to severe lymphoedema is estimated to affect at least 1 million people in Ethiopia. Although there are encouraging responses to treatment using foot hygiene, bandaging and foot wear, woody hard fibrous nodules complicating the clinical picture in some patients are resistant to therapy. Surgical interventions to the limbs are of concern in all lymphoedema patients due to the risk of poor healing. We present our experience with a series of nodulectomies performed in Northern Ethiopia in a resource-limited setting. Podoconiosis patients with persisting significant fibrous nodules despite conventional therapy were offered limited surgical nodulectomies. These were performed under local anaesthetic. Fibrotic nodules and tumours were excised with a surgical blade, aiming for the narrowest possible base. Redundant skin was shaved off and haemostasis achieved. The area was cleaned with normal saline, dressed with sterile gauze and compression bandages applied. Wounds managed in this way healed by secondary intention. Eleven patients were reviewed and wounds cleaned and dressed on alternate days in the nearby local clinic. The end point was recorded as time to re-epithelialisation in days post surgery. Eighteen surgical nodulectomy operations were undertaken on eleven patients. All patients attended on at least one occasion for review. Average time to complete

re-epithelisation was 22.3 days (range 17-43). All wounds healed. Eight patients received oral antibiotics at the time of surgery and nine received topical antibiotics subsequently. Only two patients developed clinically relevant wound infections. In conclusion, we have demonstrated that surgical nodulectomies can be performed with satisfactory healing rates and encouraging lack of complications in a tropical resource poor setting. We hope this will provide confidence for clinicians to undertake similar life quality enhancing procedures in other settings.

240

INFLUENZA-LIKE ILLNESS IS THE MOST COMMON CAUSE OF FEVER AMONG PREGNANT WOMEN IN BLANTYRE, MALAWI

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Pregnant women are at increased risk of severe disease with influenza infection, compared to other adults. Studies of influenza vaccination among pregnant women in developed and developing countries outside of Africa demonstrate a benefit to both mothers and infants. Information about the burden of influenza in Africa is limited. We conducted an observational study of 450 pregnant women in Blantyre, Malawi. Women were evaluated every four weeks and every time they were ill. We captured information about the incidence of the clinical syndrome of influenza like illness (ILI) and also obtained malaria smears at every visit, regardless of symptoms. The definition of ILI was temperature ≥37.8 degrees Celsius or history of fever and sore throat and/or cough. The study accrued 157 person years of follow up during pregnancy and 37 episodes meeting the clinical criteria of ILI were detected. This included 5 cases of respiratory symptoms and fever and also a positive malaria smear. This represented an incidence density of 23.6 episodes per 100 person years compared to 59.9 episodes of malaria infection per 100 person years. ILI was the most common cause of fever in this cohort of pregnant women. Among 79 episodes of fever detected during active and passive surveillance, 37 (46.8%) were associated with ILI clinical syndrome and 19 (24.1%) were associated with a positive malaria smear. Most episodes of ILI occurred during the cool dry season. There was no difference in birth weight or gestational age among the infants born to mothers with or without ILI during pregnancy. However, there was a borderline significant difference in neonatal deaths among infant born to women with and without ILI during pregnancy (2.4% vs. 8.8%, p=0.08). Influenza-like illness during pregnancy is an important factor in maternal morbidity and may be a risk factor for early neonatal death. Further investigation, including confirmatory testing, is required to determine the effect of influenza on maternal and infant outcomes in Africa.

241

IMMUNO-AFFINITY APPROACHES TO ENRICH FOR PATHOGEN PROTEINS IN PLASMA COLLECTED FROM CHILDREN WITH UNDIAGNOSED CAUSES OF COMA

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Non-traumatic coma in febrile children is an important syndrome with significant mortality and residual sequelae in hospitals across Africa. Although *Plasmodium falciparum* cerebral malaria (CM) is commonly assumed to comprise a large proportion of non-bacterial cases of coma, in the last five years, 51% of comatose children admitted to Kilifi District Hospital (KDH) on the Kenyan coast have coma with no cause identified. Importantly, case fatality in children with an undetermined cause of encephalopathy is as high as 33%, considerably higher than for known causes of coma. Autopsy studies in Malawian children with clinically-defined CM show an alternative diagnosis in approximately 23%. Bacterial cultures have high specificity for bacterial meningitis but inherently low sensitivity, exacerbated by antimicrobial use prior to sampling. Viral agents are rarely identified in resource poor settings as the cost of identifying the causative agents is prohibitive. Knowing the causative agent may help inform preventive vaccine strategies as well as prioritize clinical trials of antivirals or disease-modifying agents in some children with poor predicted outcomes. We have isolated immunoglobulins from plasma samples from children with confirmed bacterial, viral and malarial causes of coma. Preliminary results indicate that immunoglobulins isolated from children with cerebral malaria bind proteins extracted from a laboratory isolate of *Plasmodium falciparum* whereas immunoglobulins isolated from children with acute bacterial meningitis do not. We are now exploiting the technique to enrich for pathogen proteins in plasma of children with undiagnosed causes of coma to try and establish cause.

242

WILLINGNESS TO PARTICIPATE IN MASS MALARIA VACCINATION PROGRAMS AMONG WOMEN ATTENDING AN INFANT WELFARE CLINIC IN LAGOS, NIGERIA

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With the discovery of the malaria vaccine come plans to roll out vaccination programs in malaria endemic regions. Nigeria currently has a huge public health burden as a result of malaria and may qualify for this effort. This study was conducted to assess the willingness to participate in malaria vaccine use among mothers in Lagos, Nigeria. Self administered questionnaires were completed by 256 respondents with age ranging from 17 to 44 years. The questions were in Pidgin English which is an adulterated form of English language widely spoken among the respondents. SPSS version 10 data editor was used to analyze data. Uni-variate odds ratios and 95% confidence intervals (95 % CI) were used to evaluate the correlates of willingness to participate (WTP).35% of the respondents reported that they will be willing to have a malaria vaccine. Greater willingness was associated with prior experience of severe malaria (OR = 1.33, 95% CI: 1.02-1.73), involvement in high risk sexual behaviour (OR = 1.55, 95% CI: 1.15-1.72), higher levels of awareness about malaria (OR = 1.57, 95% CI: 1.44-1.78) and government sponsored incentives (OR = 1.49, 95% CI: 1.12-1.92). Decreased WTP was associated with concerns about physical harm to the children (OR = 0.42, 95% CI: 0.11-0.64), social stigmatization (OR = 0.31, 95% CI: 0.22-0.77) and multiple doses of vaccines (OR = 0.61, 95% CI: 0.46-0.93).The level of WTP recorded indicates that much work still needs to be done in the area of educating potential recipients of the vaccine. Government or industry-sponsored Incentives for would-be subjects could also encourage uptake among mothers and children.

243

DEVELOPMENT AND VALIDATION OF A QUANTITATIVE PCR (TAQMAN) ASSAY FOR DETECTION OF ACUTE EARLY CASES OF PATHOGENIC *LEPTOSPIRA* SPECIES IN CLINICAL SAMPLES FROM THAILAND

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Leptospirosis is a potentially fatal zoonotic illness in man if not detected at an early disease stage. The current reference methods for diagnosis - the microscopic agglutination test (MAT) and IgM ELISA - are time consuming,

and not sensitive enough to detect early cases. To improve methods for early detection, we developed a highly sensitive and specific field-ready PCR assay using freeze-dried lipoprotein reagents. Using clinical samples from a previous fever study conducted in Thailand, we evaluated the utility of the PCR assay, compared to the sensitivity and specificity of the standard MAT and ELISA methods, for detecting pathogenic species of *Leptospira*. Sample preparation with a single centrifugation step, and without the need for sample filtration was used. A total of 105 blood samples were tested in our PCR assay and the results were compared with those of the MAT or IgM ELISA methods. PCR identified leptospirosis positive cases from 38 (36%) blood samples, whereas only 14 (13%) and 5 (5%) of cases were positive by MAT IgM ELISA, respectively. PCR detected the presence of leptospirosis infection in 12 patients before development of leptospira-specific antibodies. The assay detected as few as 10 spirochetes per ml, permitting identification of leptospirosis in the early asymptomatic phase of infection. The assay is specific for detecting only pathogenic *Leptospira*, and does not cross-react with DNA from murine or human hosts, other microbes, or non-pathogenic *Leptospira* species. The assay is a rapid, highly sensitive and specific method for detecting early, acute leptospirosis cases otherwise undetectable by MAT & ELISA.

244

PENTALINON ANDRIEUXII MUELL-ARG (APOCYNACEA) ROOT EXTRACT IS EFFECTIVE IN TREATMENT OF CUTANEOUS LEISHMANIASIS CAUSED BY *LEISHMANIA DONOVANI*

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Mayan traditional medicine uses the roots from the plant *Pentalinon andrieuxii* Muell.-Arg. (Apocynaceae) to treat many different illnesses. In this work, we report that a root extract from this plant displayed strong leishmanicidal activity when tested *in vitro* against intracellular and extracellular parasites. This root extract was found to be more effective than the first-line drug, Pentostam, which results in toxic side effects and poor patient compliance. *In vivo* work using the root extract to treat *Leishmania donovani*-infected Balb/c mice showed a significant reduction in parasite loads in the liver and spleen. This extract may be considered as an inexpensive and effective alternative to treat visceral leishmaniasis provoked by infection with *L. donovani*. Authors declare they do not have a conflict of interest.

245

NEUROIMAGING IN RETINOPATHY NEGATIVE CEREBRAL MALARIA

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Our objective was to determine if there were MRI findings associated with the adverse outcomes of mortality or neurologic morbidity in children with retinopathy negative cerebral malaria. We reviewed MRI scans performed on a 0.35T magnet in 45 children with this syndrome to describe their neuroimaging findings. Univariate and multivariate analyses were performed to determine if there were MRI findings associated with adverse outcomes. Three (6.7%) children died and 8/42 (19.1%) of survivors had neurologic sequelae. On univariate analysis, all children who died or had neurologic sequelae had T2 weighted imaging abnormalities in cortical gray matter, almost always (10/11 or 90.9% of the time) associated with cortical DWI abnormalities. Children without cortical DWI abnormalities were extremely likely to survive without neurologic sequelae. White matter abnormalities did not affect the odds of having an adverse outcome. On multivariate analysis, no neuroimaging findings were associated with mortality. Cortical DWI abnormalities, focal cortical changes, cortical T2 signal abnormalities, and increased brain volume were significantly associated with neurologic morbidity in survivors. The highest odds of morbidity occurred in children with cortical DWI abnormalities. Due to small patient numbers these findings must be considered preliminary. Cortical DWI abnormalities and increased brain volume may be associated with an increased odds of an adverse neurologic outcome in retinopathy negative cerebral malaria survivors. MRI abnormalities in these children may indicate potential therapeutic targets for future clinical trials in children with retinopathy negative cerebral malaria.

247

STRANGERS IN A STRANGE LAND: SUICIDES AMONG HER MAJESTY'S BRITISH TROOPS IN INDIA, 1862-1871

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Researchers have long noted the impact of unfamiliar environmental conditions upon military personal. Torrential rain, humidity, tropical disease, and homesickness, for example, historically have exacted an important toll on the combat effectiveness of troops in the field. This has perhaps been especially true of troops who, raised in the moderate climates of Western Europe or North America, found themselves assigned to far-flung_and unfamiliar--outposts in Indochina, Africa, or India. As recent data from troops returning from Afghanistan has suggested, suicide may be the unfortunate outcome of such conditions. Suicide, however, was no less issue among British colonial troops in mid-nineteenth century India. Drawing from contemporary statistical data as well as first-hand accounts of colonial troops, this study will examine the impact of unfamiliar tropical conditions upon Her Majesty's British troops in India between 1862 and 1871.

248

BRUCELLOSIS IN TRAVELERS AND IMMIGRANTS: A TEN-YEAR RETROSPECTIVE REVIEW OF ADULT AND PEDIATRIC CASES IN HOUSTON, TEXAS

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Brucellosis is one of the most common zoonoses worldwide, but a rare and notifiable disease in the United States, where the majority of cases are reported in travelers or immigrants from endemic regions, mostly Central America and the Middle East. Recent evidence suggests that early diagnosis and initiation of appropriate antimicrobials during a "treatment window" are associated with increased cure rates. This study was undertaken to describe the epidemiology of brucellosis in Houston, Texas, and identify clinical and laboratory findings that could serve as early diagnostic clues. In this retrospective case-series, we identified patients diagnosed with brucellosis between 1/2000 - 12/2009, by searching electronic medical records for encounters with the ICD-9 code 0.239, and reviewing microbiology records for positive cultures at two University Hospitals that serve a large immigrant population. Cases were defined as those with a positive blood culture for *Brucella sp.*, or those with a serum agglutination titer ≥1:80, along with an epidemiologic risk factor and clinical presentation consistent with brucellosis. We reviewed patient demographics, exposure history, clinical presentation and laboratory data. Six adult and twelve pediatric cases were identified. 17/18 (94.4%) were immigrants from Central America. The most common risk factor recorded was ingestion of unpasteurized milk products (77.7%). The median age was 11 years (range: 21 months to 61 years) and 55.5% (10/18) were male. The most common clinical features were fever (83.3%), arthralgias

or frank arthritis (66.6%), hepatomegaly and/or splenomegaly (61.1%). The most common laboratory finding was elevated transaminases (ALT [median (range)]: 74 (38-616); AST: 78 (27-782) IU/L). Three adults (50%) but no children had thrombocytopenia (platelets <100,000/mcL, *P=0.025*). In conclusion, in the Southern U.S., brucellosis is an important consideration in the differential diagnosis of immigrants from Central America presenting with fever or joint complaints. Patients should be specifically asked about ingestion of unpasteurized dairy products. *Thrombocytopenia* (in adults) and elevated transaminases are the most frequent diagnostic clues.

249

ESTIMATING LEPTOSPIROSIS INCIDENCE USING HOSPITAL-BASED SURVEILLANCE AND A POPULATION-BASED HEALTH CARE UTILIZATION SURVEY IN TANZANIA

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The incidence of leptospirosis, a neglected zoonotic disease, is uncertain in Tanzania and in much of sub-Saharan Africa, resulting in scarce data on which to prioritize resources for public health interventions. In this study, we estimate the incidence of leptospirosis in 2 districts in the Kilimanjaro Region of Tanzania using multipliers derived from a population-based health care utilization survey (HCUS) and cases identified through hospital-based sentinel surveillance. We conducted a population-based household HCUS in the Moshi Rural and Moshi Urban Districts in the Kilimanjaro Region from June 13 through July 22, 2011. Wards within the 2 districts were selected randomly with population-weighting; 27 households in each ward were included in the survey. Heads of household were queried about the health care seeking behavior of household members in the event of fever. Febrile illness sentinel surveillance was conducted at 2 hospitals in Moshi from September 17, 2007 through August 31, 2008. Leptospirosis cases were identified among febrile adult and pediatric inpatients using the standard microscopic agglutination test(MAT); confirmed leptospirosis was defined as a ≥4-fold MAT titer rise and probable leptospirosis as any reciprocal titer ≥800. A total of 810 households were enrolled in the HCUS and multipliers were derived based on responses to questions about health care seeking in the event of febrile illness. Of participants enrolled in fever surveillance residing in Moshi Urban and Moshi Rural, 42(7.1%) of 588 met the case definition for confirmed or probable leptospirosis. After applying multipliers to account for hospital selection, MAT sensitivity, and study enrollment, we estimated the overall incidence of leptospirosis to be ~102 cases/100,000 persons annually. In the first study of leptospirosis incidence in Tanzania, we demonstrate a high incidence. Multiplier methods, such as used in this study, may be a feasible method for improving availability of incidence estimates for neglected diseases, such as leptospirosis, in resource constrained settings.

250

IODINE DEFICIENCY IN PREGNANT WOMEN IN RURAL HAITI

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Because of the poor soil and lack of supplemental iodine, we have hypothesized that iodine deficiency in pregnancy may account for the significant developmental delays we identified in children in our rural Haitian community. We collected urine specimens for iodine levels on 26 gravid women in their late second and early third trimester from two rural villages in Haiti, La Croix and La Coup. La Croix is located in the Artibonite Valley and La Coup is a mountain village. Of the 18 women from La Croix, one half of the women had urine iodine levels of less than 150 µg/L. Of the eight women from La Coup, the median level was less than 60 µg/L. In rural Haiti, there is evidence of iodine deficiency among the pregnant women as indicated by urine iodine levels in gravid women in the second and third trimester. The deficiency is more pronounced in the mountain area. Iodine deficiency may be a significant cause of developmental delay in our villages in rural Haiti.

251

IMPACT OF ANTI-RETROVIRAL THERAPY ON HELMINTHS PREVALENCE AND WORM LOAD IN HIV INFECTED PATIENTS IN GABON

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There is anecdotal evidence for both a reduced prevalence of intestinal helminth infection and worm burden, in HIV positive patients receiving anti-retroviral treatment (ART). The aim of this study is to describe possible differences in both prevalence and worm burden of geo-helminthic infections between HIV infected patients who receive ART, versus those who are not. Furthermore, this study aims to identify possible anthelminthic effects of respective anti-retroviral drugs. ART reduces both prevalence and worm burden in HIV infected patients, irrespective of the level of immune restoration. If so, this effect is due to mitochondrial toxicity of drugs leading to damage of helminthic mitochondria. This is a cross-sectional observation study in HIV patients on ART vs ART naïve patients, matched for CD4 counts, attending the HIV clinic (the 'Centre de Traitement Ambulatoire') Lambaréné, Gabon. Furthermore, a prospective observation study is performed in ART naïve individuals who start ART. Patients are analyzed for geo-helminth infections, microfilariasis and schistosomiasis. Worm larvae and adult worms are preserved in formaline for subsequent electron microscopic analysis for mitochondrial toxicity. At this moment, a total of 177 patients are included in the study, 165 in the cross sectional study and 12 in the prospective study. Demographic characteristics for the different patient groups are statistically not different. The prevalence of *Loa loa* infection is significantly lower in the patient group taking ART (11% vs. 33%, p=0.02). Also, there is a trend towards lower prevalence rates of *S. haematobium* (4% vs. 13%, p=0.18) and overall helminth infection (28% vs 60%, p=0.443) in the patients on ART. In conclusion, preliminary results of our study suggest a direct effect of ART on *Loa loa* infection, and possibly on other parasites. Whether this effect is caused by mitochondrial toxicity will be tested at a later stage of this study. Inclusion for this study is still ongoing.

252

LYMPHATIC FILARIASIS RELATED LYMPHEDEMA: A SYSTEMATIC REVIEW OF INTERVENTIONS TO PREVENT OR REDUCE MORBIDITY

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The Global Program for the Elimination of Lymphatic Filariasis (GPELF) was initiated by the WHO in 2000 with its 'first pillar' the goal of interrupting transmission of the disease by 2020. A second pillar aims to prevent or alleviate disability from chronic disease for the approximately 120 million